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With research for the success of Darányi Program

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CONFERENCE PROGRAM

PLENARY SECTION 7th OF NOVEMBER (THURSDAY)

Chair: Gyula VATAI, Csilla MOHÁCSI-FARKAS			Aula (Díszterem)
10:00	Opening/Megnyitó	**Zsolt ROSTOVÁNYI**	
10:15	Ceremonial Opening/Ünnepi Megnyitó	**Endre KARDEVÁN**	
10:30	Food Physics in the Field of Cocoa and Chocolate	**Reinhold SCHERER**	
11:00	Biotechnology, biosafety and diversity of yeasts *Saccharomyces cerevisiae*: Can all this be managed?	**Peter RASPOR**	
11:30	Physical properties of foods - 22 years with prof. András Fekete	**József FELFÖLDI, László FENYVESI**	
12:00	Extracts from the research of the National Collection of Agricultural and Industrial Microorganisms	**Gábor PÉTER**	
12:20	Emerging technologies for food waste processing: ultrasound assisted extraction of bioactive compounds from wine lees	**Mladen BRNCIC**	
12:40 - 14:30	**Networking luncheon + visit to Departments**		**Aula**
Chair: Peter RASPOR, József FARKAS			Aula (Díszterem)
14:30	Food quality and globalisation	**Zoltán SYPOSS**	
14:45	Strategic dilemmas of Hungarian food economy in globalising world	**Zoltán LAKNER**	
15:00	Changes in some ripening characteristics of semi-hard cheese as an effect of high hydrostatic pressure	**Klára PÁSZTOR-HUSZÁR**	
15:15	Applied research on fine vinaigrette emulsion production by special membrane technique	**András KORIS**	
15:30	Characterization of two bactericoins active against *Listeria* species produced by *Lactobacillus plantarum* and *Lactobacillus sakei*	**Tekla ENGELHARDT**	
15:45	Effects of different substrates on growth of *Shewanella xiamenensis*	**Attila SZÖLLŐSI**	
15:45 - 16:15	**Coffe break**		**Aula**
Chair: Reinhold SCHERER, József FELFÖLDI			Aula (Díszterem)
16:15	Selection of the most suitable starter culture for low-fat set-type yogurt with microbial transglutaminase	**Lívia DARNAY**	
16:30	The effects of TiO2 for ultrafiltration parameters by whey separation	**Zsolt László KISS**	
16:45	Consumers' opinion about their own food - purchasing habits- experiences of a quantitaive study	**Barbara BÓDI**	
17:00 - 19:00	**Poster presentations, Chair: Ágoston HOSCHKE**		**Corridor "K"**
19:00	**Dinner and wine tasting**		**Aula**

SECTION 1 8th OF NOVEMBER (FRIDAY)

Agricultural- and food-engineering; Biotechnology and fermentation processes
Agrár- és élelmiszertudomány; Biotechnológia és erjedés ipar

Chairs: Hodúr Cecília, Vatai Gyula

9:00	Különböző előkezelések hatása a szárítási jellemzőkre és a fagyasztva szárított meggy minőségére	**Antal Tamás**	**Room K3**
9:15	Gumicukrok reológiai viselkedésének modellezése	**Csima György**	
9:30	Sárgarépa (*Daucus carota* subsp. *sativus*) mechanikai és ízjellemzőjének változása nem ideális tárolsái körülmények között	**Kaszab Tímea**	
9:45	Anyagátbocsátási jelenségek vizsgálata meggysűrítmény kíméletes előállítása során ozmotikus desztillációval	**Rácz Gábor**	
10:00	A membrán modul vibráltatásának hatása a tejipari szennyvizek tisztítására	**Kertész Szabolcs**	
10:15	A Nagy csalán (*Urtica dioica L.*) hatóanyagainak kinyerése Soxhlet- és szuperkritikus extrakcióval	**Kőszegi Kornélia**	
10:30 - 11:00	**Coffee break**		**Aula**
11:00	Tejsavó zsírtalanításának vizsgálata membránszűréssel és más módszerekkel	**Szélpál Szilárd**	**Room K3**
11:15	Bio alakor búza (*Triticum monococcum* L) és bio alakor sör egészségvédő hatásának vizsgálata	**Fogarasi Attila-Levente**	
11:30	Must mikrohullámú kezelésének hatása az erjedés folyamatára	**Kapcsándi Viktória**	
11:45	Bioélesztő hatása a borok polifenol-összetételére	**Nyitrainé Sárdy Diána**	
12:00	Diaszűrési folyamatok modellezése és optimálása	**Kovács Zoltán**	
12:15	A vízellátás hatása a paradicsom táplálkozási szempontból jelentős beltartalmi jellemzőire	**Lugasi Andrea**	
12:30	**Poster Awards**	**Mohácsiné Farkas Csilla**	**Aula**
12:40	**Closing remarks**	**Palkovics László**	
12:50 - 14:00	**Networking luncheon**		

SECTION 2 8th OF NOVEMBER (FRIDAY)

Food chemistry, analytical chemistry, food microbiology
Élelmiszerkémia, -analitika, élelmiszer-mikrobiológia

Chairs: Stefanovitsné Bányai Éva, Maráz Anna

9:00	Baktériumok kommunikációja és annak élelmiszer-mikrobiológiai jelentősége	**Farkas József**	**Room K4**
9:15	*Lactobacillus* fajok azonosítása molekuláris módszerekkel	**Pál Károly**	
9:30	Gabonák malmi frakcióinak vizsgálata IR spektroszkópiai IR mikroszkópiai módszerekkel	**Izsó Eszter**	
9:45	Módszer fejlesztése különböző állatfajok tejének közeli infravörös spektroszkópiai felismerésére és osztályozására	**Patonay Katalin**	
10:00	OWLS alapú immunszenzor fejlesztése aflatoxin m1 kimutatására	**Szalontai Helga**	
10:15	Rövid ciklusú csíráztatás – egy új élelmiszeripari adalékanyag fejlesztése	**Berceli Mónika**	
10:30 - 11:00	**Coffee break**		**Aula**
11:00	A *Botrytis cinerea* genotípusos és fenotípusos sajátosságai kombinált membránszűréssel	**Hegyi-Kaló Júlia**	**Room K4**
11:15	Összehasonlító tanulmány: a fontosabb fitoszterinek stabilitása és lehetséges átalakulási útjai	**Haraszti Júlia**	
11:30	Biológialag aktív peptidek stabilitásának vizsgálata élelmiszermintákban	**Rapi Sándor**	
11:45	Fekete bodza (*Sambucus nigra* L.) antocianin profiljának fajtafüggése	**Szalóki-Dorkó Lilla**	
12:00	Brokkoli bioaktív komponenseinek vizsgálata a termesztési körülmények függvényében	**Nagyné Gasztonyi Magdolna**	
12:15	EZ_FAMILY - membránszűrés, és gyors mikrobiológiai vizsgálatok egyszerűen	**Sör Éva**	
12:30	**Poster Awards**	**Mohácsiné Farkas Csilla**	**Aula**
12:40	**Closing remarks**	**Palkovics László**	
12:50 - 14:00	**Networking luncheon**		

SECTION 3 8th OF NOVEMBER (FRIDAY)

Food technology; Food economy, logistics and consumers

Élelmiszertechnológia; Élelmiszergazdaság, -logisztika és fogyasztói tanulmányok

Chairs: Barta József, Lakner Zoltán

9:00	Légáramú előszárítással kombinált mikrohullámú vákuumszárítás előkezelési lépéseinek és előszárítási paramétereinek vizsgálata	**Ferenczi Sándor**	**Room K5**
9:15	Nagy hidrosztatikus nyomás kezelés hatása szárazáru jellemzőire az érlelés során	**Friedrich László**	
9:30	Sous-vide technologiával és nagy nyomással kezelt sertés karajok impedancia spektruma	**Kenesei György**	
9:45	Sertés karaj minőségi paramétereinek összefüggése a SEUROP minősítési rendszerrel és a márványozottsággal	**Surányi József**	
10:00	Ionizáló sugárkezelés alkalmazása immungyenge betegek étrendjének bővítésére	**Mohácsiné Farkas Csilla**	
10:15	Kókuszzsír és tejzsír egyes fizikai tulajdonságának változásai a keverés függvényében	**Soós Anita**	
10:30 - 11:00	**Coffee break**		**Aula**
11:00	Almatörköly hatása sütésálló lekvárok állomány-és reológiai tulajdonságaira	**Szabó-Nótin Beatrix**	**Room K5**
11:15	Magas antioxidáns tartalmú zöldségkrémek fejlesztése és humán klinikai vizsgálata	**Palotás Gábor, Lugasi Andrea**	
11:30	Élelmezéspolitikai paradoxonok	**Balogh Sándor**	
11:45	Funkcionális élelmiszerek marketing vonatkozásai	**Temesi Ágoston**	
12:00	Az agrárfelsőoktatás dilemmája	**Szendrő Éva**	
12:15	INNOCOACHING©: A vezetői coaching szükségessége a kutatás-fejlesztés területén	**Kurucz Balázs**	
12:30	**Poster Awards**	**Mohácsiné Farkas Csilla**	**Aula**
12:40	**Closing remarks**	**Palkovics László**	
12:50 - 14:00	**Networking luncheon**		

SECTION 4 8th OF NOVEMBER (FRIDAY)

Food safety and quality control; Nutrition

Élelmiszerbiztonság és minőségbiztosítás; Táplálkozástudomány

Chairs: Simonné Sarkadi Livia, Fodor Péter

9:00	A szerbiai élelmiszer feldolgozó ipar élelmiszerbiztonsági irányítási rendszereinek az elemzése	**Kovács Sárkány Hajnalka**	**Room K8**
9:15	*SMARTFRESH minőségi rendszer alkalmazása körtén*	**Hitka Géza**	
9:30	Funkcionális élelmiszerek célzott nyomonkövetésére alkalmas elektronikus rendszer kifejlesztése	**Naár Zoltán**	
9:45	*Capsicum annuum* var. *grossum* csemegepaprika fajták endofita baktériumainak izolálása és biodiverzitásának vizsgálata	**Füstös Zoltán**	
10:00	Étkezési paprikából (*Capsicum annuum* var. *grossum*) izolált potenciális endofita baktériumok élelmiszer-biztonsági vonatkozása	**Belák Ágnes**	
10:15	Kávé illetve kajszilé fogyasztásából származó klorogénsav *in vivo* anyagcseretermékei a vizeletben	**Abrankó László**	
10:30 - 11:00	**Coffee break**		**Aula**
11:00	Magyarországon termesztett kajszik (*Prunus armeniaca*) polifenol-tartalmának átfogó feltérképezése	**Nagy Ádám**	**Room K8**
11:15	Nitrit a húskészítményekben	**Zsarnóczay Gabriella**	
11:30	*In vitro* emésztési modell fejlesztése és jellemzése analitikai módszerekkel	**Juhász Ákos**	
11:45	A mesterséges transz-zsírsavak egészségügyi kockázatai	**Martos Éva**	
12:00	Hazai piacon elérhető pre- és probiotikum tartalmú élelmiszerek összehasonlító vizsgálata *in vitro* emésztési modellben	**Mikuska Kata**	
12:15	Különböző termesztéstechnológiai körülmények hatásának élelmiszerbiztonsági vizsgálata burgonyában	**Tömösköziné Farkas Rita**	
12:30	**Poster Awards**	**Mohácsiné Farkas Csilla**	**Aula**
12:40	**Closing remarks**	**Palkovics László**	
12:50 - 14:00	**Networking luncheon**		

Plenary session

CONSUMERS' OPINION ABOUT THEIR OWN FOOD-PURCHASING HABITS- EXPERIENCES OF A QUANTITAIVE STUDY

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SUMMARY
Food waste is produced through the food chain from agricultural raw material production to the households. Dominance of globalized logistics, increased expectations of retailers, changing habits of consumers and difficulties in the planning of the supply chain all contribute to the production of waste material. While losses have already been reduced significantly by developed technologies in food processing and retail, there is still an enormous amount of food waste produced by households every year. Our study endeavours to provide a comprehensive review of consumer's attitude regarding food purchasing and consumption. We used quantitative method (nationwide survey based on personal interviews) for determining consumers' standpoints concerning their own shopping habits. Data were gathered through mentioned interviews with a random sample of 1027 people, aiming appropriate representation of the Hungarian middle class. Between multivariate statistical methods we applied Pearson's chi-squared to ascertain diversity of opinion among respondents.

1. INTRODUCTION

Sustainability considerations have not been significant in the food chain for former centuries. On the other hand, agricultural and food industry technologies have developed for efficiency improvement and cost reduction purposes. Nowadays sustainability aspects have effects on all economy fields (Csutora& Kerekes, 2004; Gaál et al., 2011), thus food industry increasingly starts to focus on the environmental impact of production. While losses in developing countries occur mostly at the stage of raw material production due to obsolete technology, in industrialized countries the majority of food waste is produced by retail, catering and households according to one of FAO's study (2011) titled 'Global Food Losses and Food Waste. This paper evaluates the amount of food waste throughout the whole food chain.

While many food industrial companies could achieve significant results in waste-efficiency, consumers have not changed their buying habits towards sustainability. Reforming human mentality could be a cost effective way to have an impact to the entire food chain and could provide both short and long-time effects. Increased public awareness, thus appropriate shopping habits, use and storage of foodstuff could minimize food losses (Godfray et al., 2010), and would foster adopting sustainability approach at another food chain actors (Meulenberg et al., 2003).

The lack of awareness in buying situations is observable by investigating the amount of household-generated food waste. The first paper in this topic was written in 1975: a research was conducted by Gail and his fellow researchers involve hundreds of households. On the basis of the results it was found that an average American household produced food waste of hundreds of US dollars value a year (Gail et al., 1975). More recent studies report even worse, alarming results (Kantor et al. 1997).

Nowadays many experts, governmental and non-governmental organizations have recognized these problems and organize public awareness campaigns towards reducing food waste in several developed countries (Parfitt et al. 2010). One of the most effective social program is called "Love Food Hate Waste" and launched in the United Kingdom in 2007: according to its' report, a significant reduction (18 %) has been reached in the amount of food waste produced by the analyzed households (www.lovefoodhatewaste.com).

The initial step of designing a campaign to affect consumer habits ought to be an analysis of consumer attitudes and present practices. This study aims to help this process by providing an evaluation of some of the basic attitudes in regard to sustainable consumption.

2. MATERIAL AND METHODS

During the research planning, we aimed to gather appropriate data to represent the middle class of the Hungarian population, but paid attention to gather data from all demographic groups. According to our previous experiences in food related topics, personal interviews result in more relevant data than any other (e. g. telephone assisted or online) methods. The questionnaire based survey is a useful tool for primary data collection. Questionnaire creation is an immensely long process. It demands expertise in need to integrate professional considerations, circumstances of surveys and conditions of data processing (Hajdu-Lakner, 1999). Quantitative studies based on personal interviews require a relatively high budget. We received some help from another research programme, and had the opportunity to integrate our questions in a large-scale survey related food safety in 2013.

In the study, two opposite statements were applied with an attitude scale (5-point Likert scale) to reveal consumer's food-shopping habits:

- Statement A: I usually buy appropriate amount of food and consume everything
- Statement B: I usually buy too much food in the supermarket, I have to dispose the excess

Previous to the survey the entire questionnaire was tested by consumers and experts (n=12). As a result of the pilot survey we could refine the questionnaire and determine the average completion time, which provided important information about the technical realization of the research procedure. The personal interviews were conducted at traffic interchanges of Hungarian cities in the first half of 2013. At the end of data gathering procedure, a n=1027 sample size was achieved, and of the interviews were suitable for statistical analysis with SPSS program package. Data were analyzed by descriptive and multivariate statistical methods.

3. RESULTS

3.1. Profile of respondents

Demographic variables can be used to differentiate consumer groups. The parameters we recorded during the survey are summarized in *Table 1*.

Table 1: Content of the consumer sample

Gender				
Male: 46,51%			Female: 53,49%	
Age				
Aged under 25 years: 47,74%	Aged between 26 and 35: 18,79%	Aged between 36 and 49 years 14,17%	Above 50 years of age: 19,30%	
Habitation				
Budapest: 39,05%	Other city: 48,58%		Village: 12,37%	
Qualification				
Elementary: 15,07%	High-school graduation: 40,04%		Degree: 44,89%	
Level of income				
High: 1,11%	Above average 9,78%	Average: 53,63%	Below average: 23,59%	Low: 11,90%
Number of people living in the same house				
One person: 11,42%	Two people: 21,99%	Three people: 23,36%	Four people: 26,11%	Five people: 17,10%
Who does regularly the food shopping in your household?				
You: 32,46%	Another person: 19,22%		Together: 48,32%	

3.2. Food-purchasing habits

The participants' opinion about their own food purchasing attitude was not absolutely unified. Results of quantitative study indicate that 'buying as much food as reasonably necessary' is determinative aspect of lifestyle in spite of it consumer behaviour is occasional different – because of different influential factors – in real shopping situation.

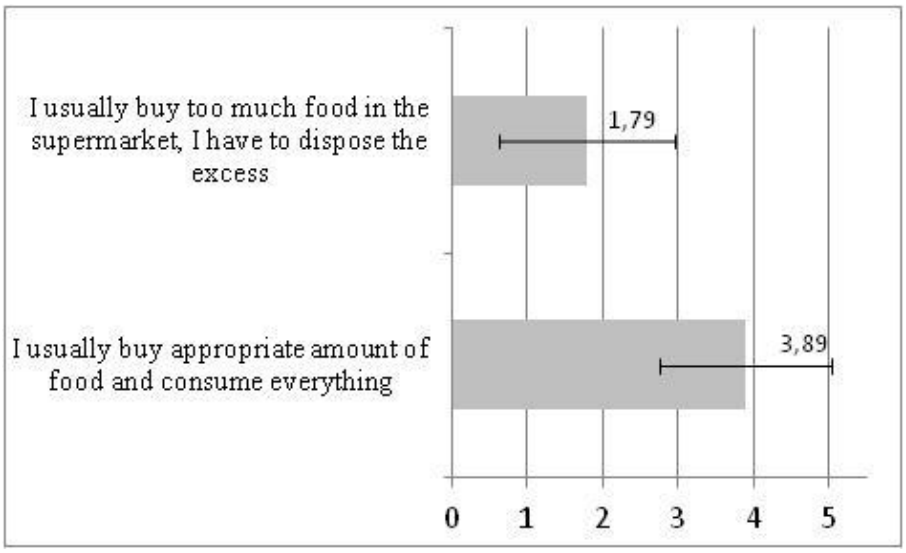

Figure 1: Consumers' opinion about their own attitude in buying situation

The crosstab analysis was done only for that subset of the sample that regularly manages food shopping in the household (32,46%). According to crosstabs results, qualification ($P<0,01$) and level of income ($P<0,01$) are significant variables in case of both investigated statements, but no differences observed by demographic segmentation of gender, age, habitation and number of people in a household. In this paper we present only the results of comparing "qualification groups" (Figure 2). Generally, we could point to that majority of the respondents claimed to be aware about their shopping habits. In the same time, the results indicate that people with lower education tend to be more extremists in both directions than people of high school education. While some part of them seemed careless about purchased quantities, the careful persons were found in a surprisingly big number in their ranks. People with higher education tend to be aware in general, but there is a relatively low share of them that care very much about the problem (compared to the other two groups). This phenomenon also related with financial status of respondents, as further crosstab analyses revealed. This is consequent to the finding that level of education correlates to and level of income.

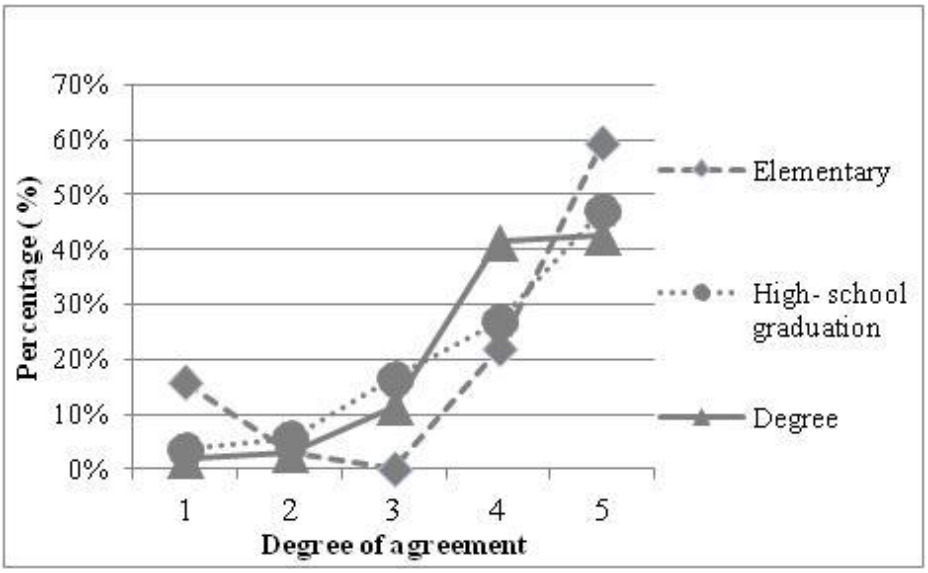

Figure 2: Crosstab of *Statement A* by "qualification groups"

4. CONCLUSIONS

According to the statistical analysis we can see that attitudes of the average consumer fit well to the expectations in regard of sustainable consumption. This let us to conclude that not the attitudes but the habits directly should be focused in a potential campaign. It was an interesting finding that high-educated people admit to waste more food than high school educated persons. In the same time we can presume that people with lower education are not careful about shopping exclusively due to sustainability – low income is also a factor to prevent excess amount of food.

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EMERGING TECHNOLOGIES FOR FOOD WASTE PROCESSING: ULTRASOUND ASSISTED EXTRACTION OF BIOACTIVE COMPOUNDS FROM WINE LEES

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Pierottijeva 6, Zagreb 10000, Croatia, mbrncic@pbf.hr

Food wastes by definition are remains or residues of high organic load that arise from food processing of raw materials under various conditions and technologies within food industry. Final formation or shape of food waste is mostly heterogeneous liquid, semi-solid or solid form. Those materials are sources of various important compounds and are taken into consideration for a long time to be processed and used due to environmental reasons but also because of re-usage in food industry as a potential raw material, pharmaceutical industry and chemical industry as well. Easy accessible and reachable food wastes could be potentially important part for obtaining valuable compounds in mentioned industries because of recovery using existing methodologies but also with promising innovative and emerging both thermal and non-thermal technologies. Those technologies are Hi-Intensity ultrasound (HIU); High hydrostatic pressures (HHP); Pulsed electric fields (PEF) and some others.

Extraction is a process frequently used in food industry, often based on the use of conventional methods. This kind of methods may lead to loss in bioactive compounds in final products. Consequently, the need for research of new extraction methods arises. The development of a rapid, reproducible and simple method of extraction of the majority anthocyans and phenols present in wine lees by the employment of ultrasound-assisted extraction was the aim of this work. The study has covered three possible solvents for the extraction (water, 30% ethanol and 50% ethanol), ultrasonic amplitude (60% and 90%) and the extraction time (4, 8 and 20 minutes). Under the optimum conditions of the method developed, 50% ethanol is employed as solvent, ultrasonic amplitude 60% and an extraction time of 20 minutes, both for anthocyans and phenols. The anthocyans extracted have been analyzed by HPLC with UV/Vis PDA detection and using monolithic columns for the chromatographic separation. Results indicate that ultrasound-assisted extraction can greatly improve the extraction of bioactive substances, achieving higher efficiency and shorter reaction times at low or moderate costs.

SELECTION OF THE MOST SUITABLE STARTER CULTURE FOR LOW-FAT SET-TYPE YOGHURT WITH MICROBIAL

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SUMMARY
Our research target was to find the most suitable liophylised DVS yoghurt culture to complete the action of microbial transglutaminase. The selected 4 different DVS cultures were manufactured by Chr. Hansen a well-known company in dairy industry. Low-fat set-type yoghurt was produced from 1.5% UHT bovine milk as the enzyme previously proved to be capable to substitute fat, resulting a product equivalent with average plain yoghurts in Hungary. Several methods were used to evaluate the combined effect of definite DVS culture and enzyme: observation of pH decreasing tendency, sol-gel transformation followed by rotational viscosimeter. Final products were compared according to gel strength, whey separation, dry matter content and sensory evaluation. The best products were further analysed during a 4-week long storage.

1. INTRODUCTION

Microbial transglutaminase (E.C. 2.3.2.13) belongs to the transferase types of enzymes, which is center of researched for new kind of stirred (Sári, Koncz, & Guyot, 2006) and set-type (Yüksel & Erdem, 2010, Sanlı et al., 2010) yoghurts. The enzyme can improve texture and decrease syneresis as it makes polymerised protein molecules build up of ε-(γ-glutamil) lisine bonds with cross-linking. Nowadays mTG can have a great importance in low-calorie diet as it is capable to enhance the quality of low-fat (Guyot & Kulozik, 2011) or even non-fat yoghurt (Ozer et al., 2007). As it seems to be already manageable to make delicious low-fat yoghurt with mTG it is still unknown which commercial starter culture matches with it the best. According to our best knowledge there was no research focusing on selecting the most suitable starter culture for producing low-fat set-type yoghurt with mTG. Our research has the aim to complete this lack of knowledge.

2. MATERIALS AND METHODS

We used in the experiments, **four types of commercial starter cultures**, YF-L811, YC-X11, YC-180, probiotic ABT-1 (Chr. Hansen A/S, Denmark). YF-L811 produces yoghurt with very high body, very mild flavour and very low post acidification. YC-X11 is suitable to make high body, mild flavour yoghurt with low post acidification. YC-180 makes medium flavour, high viscosity yoghurt with medium post acidification. The ABT-1 culture is declared as probiotic and produces a yoghurt with high body, mild flavour, minimal post-acidification according to the manufacturer's data. **Microbial transglutaminase** derived from Activa YG (Barentz Hungary Ltd., nominal activity: 104 U/g protein) was used simultaneously with starter culture adding 0.3 g powder to 1 liter milk. The 1.5% fat UHT milk for making set-type yoghurt was manufactured by Alföldi Tej Ltd. **Fermentation** lasted until pH 4.7 at 43°C, which was reached during 3 hours. Following fermentation the samples were put into a 4°C **fridge for a single night**. Texture profile analysis and sensorial analysis were carried out on the following day according to detailed description below. The samples of the 4-week-long storage test were measured on a weekly basis.

The **pH** was measured every 30 minutes with Testo 206 portable pH measuring equipment (Testo AG., Germany) until pH 4.9, after wards every 15 minutes until pH4.7 was reached.

The **structure of yoghurt** was measured with TA. XTPlus (Stable Micro Systems, Great Britain). Data evaluation was done with the software of the instrument called Texture Exponent 32 after ageing overnight. During the storage period yoghurts were measured weekly. As yoghurt belongs to the shear thinning materials, **viscosity parameters** can be described with the apparent viscosity features of non-newtonial fluids. The shear stress was measured with Rheomat 115 (Contraves, Switzerland) rotational viscometer using the conical measure head type 145. The samples were mashed through a sieve (mesh diameter: 0.80mm) and tempered to 20°C before measurement. Every measurement was done in duplicate with 100g of sample. Shear stress (τ) was calculated from the read values of the instrument (a) multiplied with the factor of the measure system type 145 (b). **After ageing** overnight the samples were **evaluated with a trained panel** (9-10 members) focusing on flavour, taste and texture. The attributes were ranked on a 0-100 score scale compared to control sample, which was fixed at the exact middle of the scale. During the storage period yoghurts were presented weekly.

Whey separation was examined after ageing. The samples were weighed and then left to drain on Büchner funnel for an hour. The volume of the collected whey was determined with measuring cylinder. The result were converted into ml whey/100 g product/hour. During the storage period yoghurts were measured weekly.

3. RESULTS AND DISCUSSION

3.1. Selecting the most suitable starter culture for low-fat set-type yoghurt treated with mTG

All **starter cultures reached pH 5.0 after 120 minutes**, among them YF-L811 and YC-X11 followed same profile as shown on Fig. 1. After 2 hours the differences are visible: YC-180 and YC-X11 produced enough lactic acid to meet the requirement (pH 4.7) in half an hour, while the others (YF-l811, ABT-1) needed an hour to reach that point. These results suggest to use YC-180 or YC-X11 to be time efficient.

The **effect of mTG** can be realised **following the gel strength** of each product as shown on Fig. 2. Gel strength was slightly enhanced by microbial transglutaminase, when using ABT-1 starter culture, but it greatly effected the body of yoghurt made with YC-180 and using mTG can be considered by YF-L811 and YC-X11 as well.

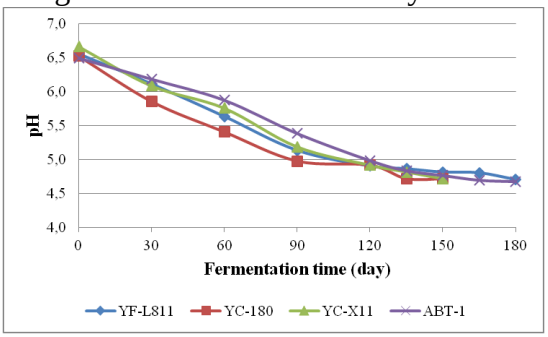

Figure 1: Changes in pH during lactic acid fermentation

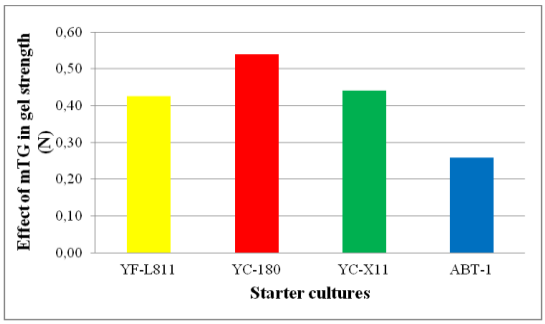

Figure 2: Effect of mTG in gelstrength depending on starter culture

As seen before in pH, YC-180 and YC-X11 has similar characteristics regarding their **inner friction properties** too as shown on Fig. 3. The two other cultures (YF-L811, ABT-1) responded to increasing shear stress in same way making parallel curves, but YF-L811 tended to be less resistant.

According to our results the less **difference in whey quantity** between enzyme treated and non-treated yoghurt was observed by probiotic ABT-1, less than 2 ml whey/g yoghurt/hour (Fig. 4.). If we made yoghurt handled using mTG then 8, 14 and 18 ml whey remained in yoghurt by YF-L811, YC-180 and YC-X11 accordingly. More than 10 ml of whey/g yoghurt/hour incorporated by mTG is a very promising sign for manufacturers.

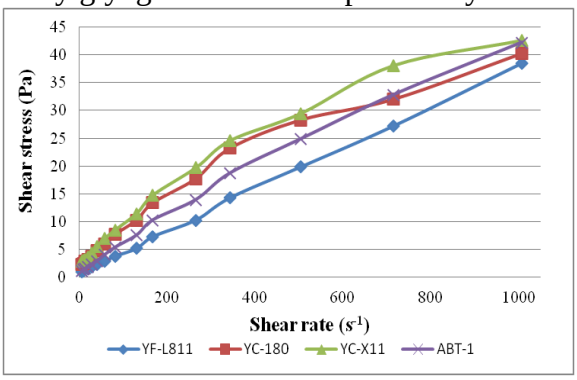

Figure 3: Changes in shear stress with different starter cultures

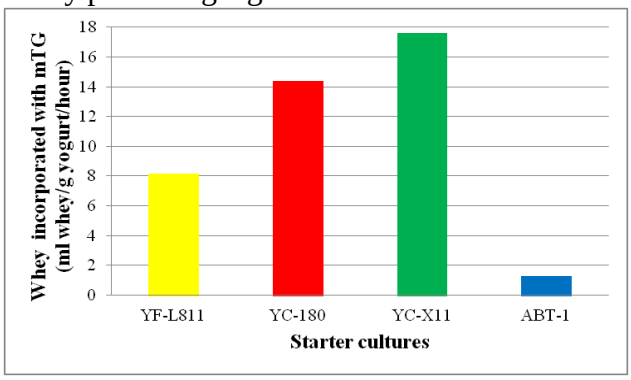

Figure 4: Changes in whey incorporation with different starter cultures

Our trained sensory panel revealed that yoghurt made with ABT-1 starter culture and mTG is the best pairing having the highest scores for nearly all attributes of yoghurt as seen on Fig. 5. We should highlight that YF-L811 were also favourable with mTG and had more pleasantly sour taste than ABT-1. Fig. 5. also shows that YC-X11 was extraordinary good in odour, structure and spoonability as YC-180 was, although YC-180 was more acceptable mainly because of taste.

According to our comparison YC-180 was selected to make low-fat set-type yoghurt with mTG as it led to fast acidification, contributed to the highest gel strength, to an advanced level of whey retention and to the right spoonability and flavour.

3.2. Storage test of low-fat set-type yoghurt treated with mTG using yc-180

According our observations see on Table 1. the **lactic acid bacteria were still active** at the low temperature of fridge (4°C) as pH lowered week by week. In the same time more and more whey was released. Under pH 4.5 the amount of separated whey tripled. The enzyme made the gel firmer every week until 21th day, but then the structure got rigid. The effect of mTG is also visible in the **results of sensory analysis** (see on Fig. 6.) as the harder the yoghurt was the higher spoonability, more porcelain structure and less smoothness were developed with time and smoothness declined.

Table 1: Changes of pH, whey separation and gel strength with storage time

Storage time (day)	pH	Whey separation (ml whey/ g yoghurt/hour)	Gel strength (N)
1	4.63	9.58	1.00
7	4.55	13.04	1.02
14	4.48	36.75	1.06
21	4.41	36.36	1.08
28	4.41	39.87	0.27

The **panellists liked the 21-day old yoghurt** in the same way as at first day (see overall acceptance scores) and they even gave higher scores for odour. In case of looking for a well spoonable yoghurt, which is even smoothie such low-fat set-type yoghurt should be consumed in 2 weeks time.

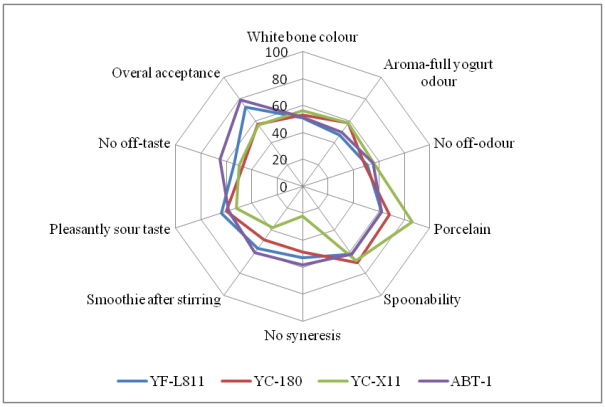

Figure 5. Changes of sensorial properties with different starter cultures

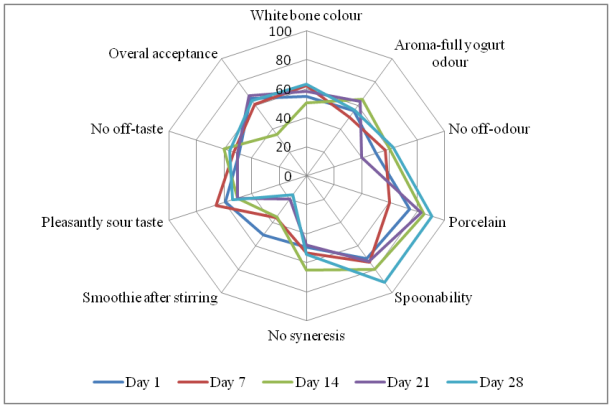

Figure 6. Changes of sensorial properties during storage time

4. CONCLUSIONS

Our research results indicate that the **YC-180** starter culture can be a good choice for making 1.5% set-type yogurt if we use **0.3 w/v% Activa YG** enzyme preparation as it enhances and stabilizes the texture and prevents syneresis in a very efficient way.

5. REFERENCES

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CHARACTERIZATION OF TWO BACTERIOCINS ACTIVE AGAINST *LISTERIA* SPECIES PRODUCED BY *LACTOBACILLUS PLANTARUM* AND *LACTOBACILLUS SAKEI*

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SUMMARY

Lactic acid bacteria were isolated from Portuguese fermented sausages, which were produced by natural fermentation. Lactobacillus plantarum ESB 202 *and Lactobacillus sakei* ESB 153 *produces bacteriocin. Bacteriocin production during growth was monitored against Listeria monocytogenes (serogroup IIb) and Listeria innocua NCTC 11288. Effect of enzymes, temperature, pH, surfactants and protease inhibitors on bacteriocin activity was determined.* ESB 202 *and* ESB 153 *bacteriocins were produced at highest level after 15-18 hours against L. monocyotogenes and L. innocua. Bacteriocins produced by Lb. plantarum* ESB 202 *and Lb. sakei* ESB 153 *proved to be stable.*

1. INTRODUCTION

Lactic acid bacteria (LAB) strains could be used in fermented meat products as starter cultures. Application of LAB could inhibit the foodborne pathogens by acid production and pH reduction, as well as bacteriocin production (Dortu et al., 2008). *Lactobacillus plantarum* ESB 202 and *Lb. sakei* ESB 153 were previously isolated from Portuguese fermented sausages. *Lb. sakei* is one of the most important species of the starter cultures used for fermented sausages production (Dortu et al., 2008).

Listeria monocyotgenes is a foodborne pathogen, which frequently occur in meat products; unfortunately *Listeria* species are able to grow at refrigerator temperature. Numerous scientific articles discuss the possibility to inhibit *L. monocyotgenes* by LAB (Balciunas et al., 2013; Martinez et al., 2013; Rodríguez et al., 2007). The aim of our study was to characterize the anti-listeria bacteriocins produced by *Lb. plantarum* ESB 202 and *Lb. sakei* ESB 153.

2. MATERIALS AND METHODS

2.1. Bacteriocin production during growth and characterization of bacteriocin

MRS broth was inoculated with 1% (v/v) of an overnight culture of each LAB and incubated at 30 °C. Changes in pH and optical density (OD) (600 nm) were recorded every hour, for 24 h. Bacteriocin activity (AU/ml) in the cell-free supernatant was recorded every 3 h for 24 h, as described by Van Reenen et al. (1998).

Effect of enzymes, temperature, pH and detergents on bacteriocin activity was done as described previously by Albano et al. (2007).

Listeria monocytogenes (serogroup IIb; isolated from cheese) and *L. innocua* NCTC 11288 were used as target strains for all of the measurements.

2.2. Cell lysis

Twenty millilitres of a bacteriocin-containing cell-free supernatant (25600 AU/ml, pH 6.0) was filter-sterilized and added to 100 ml of early exponential phase (3.5-h-old) cultures of *L. monocytogenes* and *L. innocua*, respectively. Optical densities measured at 600 nm were taken every hour, for 11 h.

3. RESULTS AND DISCUSSION

3.1. Bacteriocin production during growth and characterization of bacteriocin

Lactobacillus plantarum ESB 202 produced the bacteriocin at a highest level (25600 AU/ml) against *L. monocytogenes* after 15 hours and against *L. innocua* after 18 hours. In case of *Lb. sakei* ESB 153 the maximum level (25600 AU/ml against *L. monocyotgenes* and 12800 AU/ml against *L. innocua*) was observed after 15 and 18 hours (Fig. 1A-B). The maximum bacteriocin activity level was recorded when the pH was around 4.3. The pH was decreased from 6.3 to 4 by *Lb. plantarum* ESB 202 and from 6.3 to 3.9 by *Lb. sakei* ESB 153. In both cases, the cell density was increased in the same way. Similar results were recorded by Dortu et al. (2008) and Martinez et al. (2013).

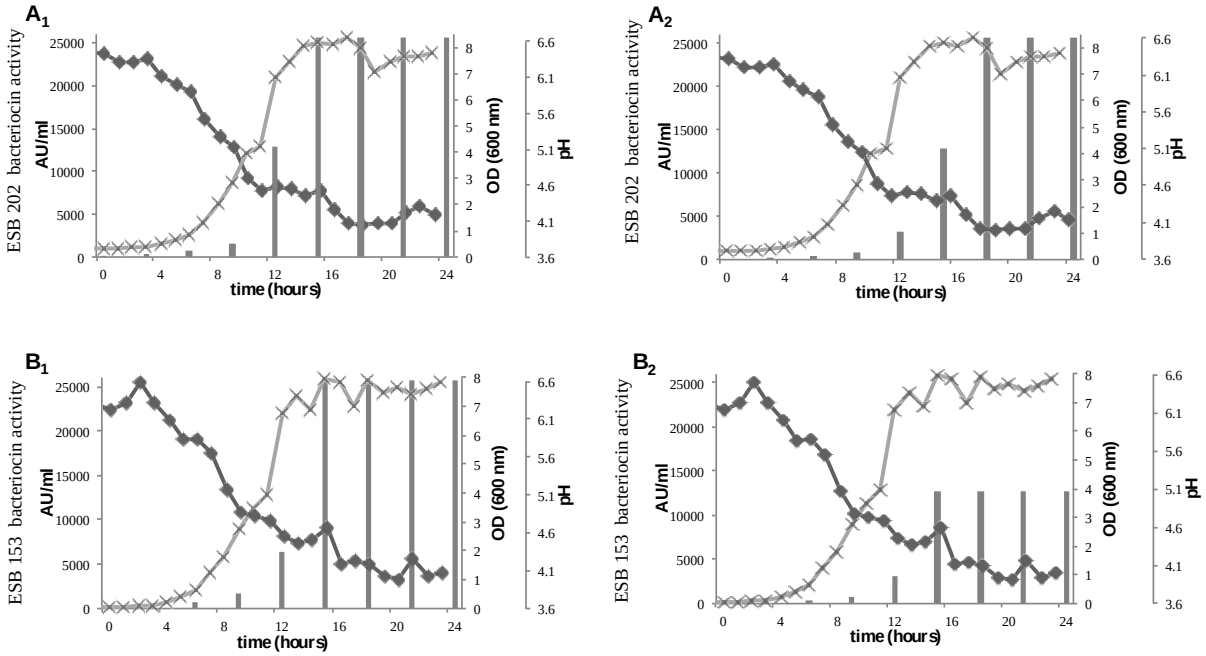

Figure 1: Production of bacteriocins ESB 202 (graph A) and ESB 153 (graph B) in MRS broth at 37 °C. 1: Sensitive strain *L. monocytogenes*; 2: Sensitive strain *L. innocua*. Antimicrobial activity of cell-free supernatants showed in AU/ml (bars), OD (X) and pH (♦).

The results from the effect of enzymes, temperature, pH and detergents on bacteriocin activity are shown in Table 1. The activity of both bacteriocins was reduced after treatment at different temperatures and pH, but *L. innocua* showed to be more resistant. Treatment with detergents were different to bacteriocin produced by *Lb. plantarum* ESB 202 and *Lb. sakei* ESB 153, no change in activity was observed against *L. monocytogenes* in case of bacteriocin produced by *Lb. sakei* ESB 153. After treatment with protease K, tyrosinase, peroxidase, pepsin and trypsin complete inactivation or significant reduction in antimicrobial activity was observed. No complete inactivation was recorded when treated with catalase, which indicated that H_2O_2 was not strictly responsible for inhibition.

Table 1: Reduction of antimicrobial activity of bacteriocins produced by *Lb. plantarum* ESB 202 and *Lb. sakei* ESB 153 (expressed in percentage values) after incubation at different conditions

		L. plantarum ESB 202				*L. sakei* ESB 153			
		L. monocytogenes 1/2b-3b		*L. innocua* NCTC 11288		*L. monocytogenes* 1/2b-3b		*L. innocua* NCTC 11288	
Temperature		after 1hour	after 2 hours	after 1hour	after 2 hours	after 1hour	after 2 hours	after 1hour	after 2 hours
	4 °C	0%	0%	50%	50%	0%	0%	25%	25%
	25 °C	0%	0%	75%	75%	0%	0%	75%	75%
	30 °C	0%	0%	75%	88%	0%	0%	50%	50%
	37 °C	0%	0%	75%	75%	0%	0%	50%	50%
	60 °C	0%	50%	75%	88%	0%	0%	50%	75%
	80 °C	0%	50%	88%	94%	0%	0%	50%	75%
	100 °C	50%	88%	88%	97%	50%	94%	75%	94%
	121 °C after 15 min	97%		99%		97%		98%	
	4 °C after 1 month	0%		75%		0%		50%	
pH (after 2h, at 25 °C)	2	50%		75%		75%		75%	
	4	50%		88%		50%		50%	
	6	75%		94%		75%		75%	
	8	50%		88%		75%		75%	
	10	0%		75%		0%		50%	
	12	94%		98%		88%		94%	
Detergents (after 5h, at 30 °C)	Tween 20	75%		94%		0%		75%	
	Tween 80	75%		94%		0%		75%	
	Triton X-100	75%		88%		50%		75%	
	SDS	0%		75%		0%		75%	
	Bile salt	75%		94%		50%		88%	
	Urea	75%		94%		0%		75%	
	NaCl	0%		88%		0%		75%	
	0.1 mM EDTA	0%		88%		0%		88%	
	2 mM EDTA	50%		94%		0%		88%	
	5 mM EDTA	75%		94%		0%		88%	
Enzymes (after 2h, at 30 °C)		1 mg	0.1 mg	1 mg	0.1 mg	1 mg	0.1 mg	1 mg	0.1 mg
	protease K	100%	100%	100%	100%	100%	100%	100%	100%
	tyrosinase from mushrooms	100%	94%	100%	99%	100%	88%	100%	97%
	peroxidase	97%	88%	98%	94%	99%	50%	100%	88%
	pepsin	99%	75%	100%	94%	99%	75%	100%	88%
	trypsin	100%	98%	100%	99%	100%	98%	100%	98%
	catalase	88%	50%	94%	94%	75%	0%	88%	50%

2.2. Cell lysis

The addition of bacteriocin produced by *Lb. plantarum* ESB 202 and *Lb. sakei* ESB 153 (Fig. 2) to mid-log phase (3.5 hours old) *L. monocytogenes* and *L. innocua* cultures showed a clearly static effect. No effect was recorded in untreated (positive controls) samples in case of both *Listeria* strains.

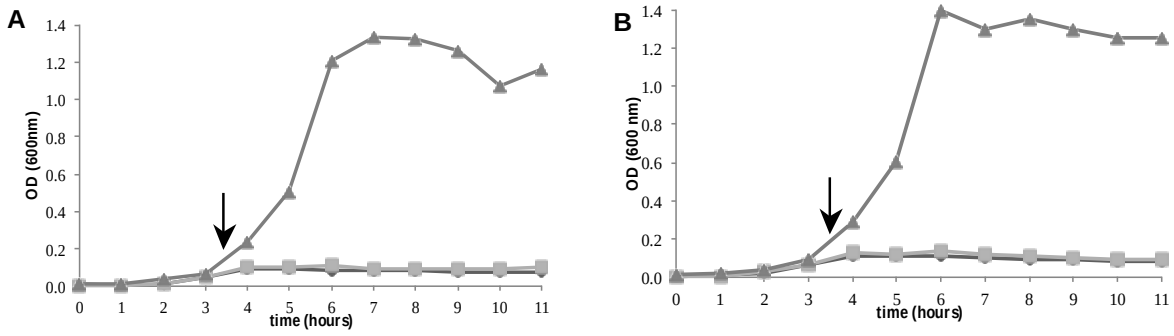

Figure 2: Effect of bacteriocins ESB 202 (♦) and ESB 153 (▪) on *L. monocytogenes* (A) and *L. innocua* (B) cultured at 37 °C. The symbol (▲) represents the growth of *L. monocytogenes* (A) and *L. innocua* (B) without added bacteriocins (controls). The arrow indicates the point at which the bacteriocins were added (3.5 h).

4. CONCLUSIONS

Most bacteriocin produced by different LAB strains showed antilisterial activity as well as lactobacilli strains (Martinez et al., 2013; Dortu et al., 2008; Rodríguez et al., 2007). Both bacteriocins - which were tested in this study – may be used in the food industry as bio-preservative agents since they seem to be stable to environmental circumstances. Further measurements are needed to understand which environmental factors may influence the bacteriocins production, which could increase the application capability of both strains.

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THE EFFECTS OF TIO_2 FOR ULTRAFILTRATION PARAMETERS BY WHEY SEPARATION

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SUMMARY

Nowadays, membrane filtration technique is a commonly used method for separation of whey. The most significant limitation factor of membrane applications is the fouling, causing flux decline, which is need to be decreased for increasing the separation efficiency. During this work the ultrafiltration (UF) of whey containing model wastewater was investigated using TiO_2 modified regenerated cellulose (RC) membranes. The changes of filtration parameters and the photocatalytic effects of the UV irradiated TiO_2 modified membrane surface on the membrane fouling were also examined. Our results showed that the water flux decreased with increasing TiO_2 layer thickness, while in the case of the whey separation, the two layers of TiO_2 resulted higher fluxes. Since ultraviolet (UV) treatment activated the TiO_2 layers, the photocatalytic oxidation resulted decreased gel-layer resistances.

1. INTRODUCTION

Membrane technology is widely used in food, chemical, and pharmaceutical industries. The major drawback of the extensive use of membranes is membrane fouling, which affects both the quality and the quantity of product water and ultimately shortens membrane life time especially when the fouling is irreversible (Chen 2006, Mingliang 2011). Therefore, membrane cleaning is an essential step in maintaining the membrane efficiencies used for the separation processes (Muthukumaran 2004). A high number of different chemical and physical cleaning methods have been using for membrane cleaning but these techniques interrupt the continuous filtration process (Muthukumaran 2005, Chai 1999). The heterogeneous photocatalysis using titanium dioxide (TiO_2) might be an other method for reduction membrane fouling because of its photocatalytic effect that can decompose organic chemicals and kill bacterias (Li 2009). Therefore, it has been applied to surface modification of several membranes (Ebert 2004). In earlier studies polyvinylidene flouride (PVDF) composite membranes were covered with different sizes of nanosized TiO_2 particles. It was found that smaller nanoparticles could improve the anti-fouling property of PVDF membrane more remarkably (Cao 2006).

Therefore, the main objective of this study was to investigate the TiO_2 nanotubes attached to the surface of RC UF membranes and their effects on the ultrafiltration parameters of model dairy wastewater treatment. Furthermore, the cleaning effects of UV radiation on the membrane structure were also investigated and how can reduce the gel layer caused resistance.

2. MATERIALS AND METHODS

Model solution was prepared from instant whey powder at 0.5 wt.%. Flat-sheet RC membrane of Amicon (PL series, Millipore) with different molecular weight cut-offs of 3 kDa and membrane effective area of 0.001734 m^2 was used.

The ultrafiltration (UF) experiments were carried out in a batch stirred cell (Millipore, Serial N°94) with a capacity of 50 cm^3. Before UF experiments, the membrane was soaked at least overnight in distilled water to remove soluble processing chemicals and to wet the membrane pores perfectly. The membranes were covered in two layers with TiO_2 nanotubes in University of Lausanne with a special method (Tetreault 2010, Horvath 2012). The initial

feed volume was 50 cm^3. The UF experiments were finished when 25 cm^3 of the total sample was filtered at 0.3 MPa transmembrane pressure. The measurements were carried out at 20 °C. Determination of the chemical oxygen demand (COD) was based on the standard method involving potassium dichromate oxidation; for the analysis, standard test tubes (Lovibond) were used. The digestions were carried out in a COD digester (Lovibond, ET 108) at 150 °C for 2 hours and then the values were measured with a COD photometer (Lovibond PC-CheckIt). The turbidity was measured with a Turbidimeter (Hach 2100N, Germany). Scanning electron microscope (SEM) images were preapared with SEM Hitachi S-4700 microscope.

Filtration laws

The filtration laws that have been widely used were cake filtration, intermediate filtration, standard pore blocking and complete pore blocking, which the cake filtration model gave the best fit (Kiss 2013). The cake filtration laws for which, formulated in terms of flux per unit time, are given in Eq. 1.

$$J = J_0(1+2K_c(A\cdot J_0)^2\cdot t)^{-0.5} \quad (1)$$

J is the flux, J_0 is the initial flux, the various k_c is the fouling coefficient, K_c is the mass transfer coefficient and A is a constant.

The selectivity of a membrane for a given solute can be expressed by the average retention (R):

$$R = \left(1-\frac{c}{c_0}\right)100\% \quad (2)$$

where c is the average concentration of the solute in the permeate phase, and c_0 is the concentration of the solute in the feed.

The volume reduction ratio (VRR) was determined as:

$$\mathrm{VRR} = \frac{\mathrm{V_{FEED}}}{\mathrm{V_{FEED}} - \mathrm{V_{PERMEATE}}} \quad [-] \quad (3)$$

where V_{FEED} is the feed volume (cm^3), and $V_{PERMEATE}$ is the permeate volume (cm^3).

3. RESULTS AND DISCUSSION

In the first series of experiments the effect of UV irradiation on membrane surface was investigated. The water fluxes of the original and TiO_2 covered RC UF membranes were examined. It was found that the untreated membranes damaged by UV irradiation (Fig 1a).

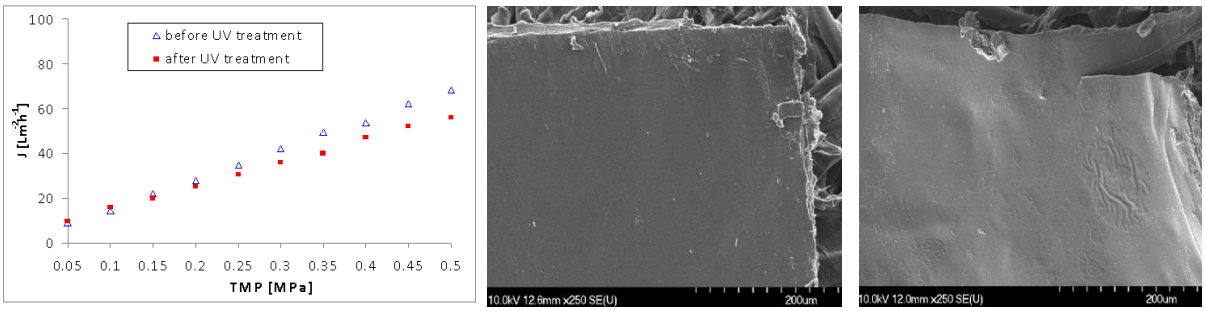

Figure 1: (a) Fluxes with increased TMP, (b) SEM micrograph of UF membrane surface, (c) SEM micrograph of UF membrane surface after 30 min UV

The homogeneous structure of the top of the membrane was changed slightly (Fig 1c) as the non-UV irradiated membrane surface (Fig 1b). Therefore, the water fluxes of the

membranes after UV irradiation (Fig 1c) resulted smaller fluxes. However, in the case of TiO_2 –covered membrane, the UV irradiation did not affect the water flux (Fig. 1a/2a). On the other hand, the TiO_2-layers fouled the membrane pores causing slightly decreased fluxes (Fig. 2a). SEM analyses were carried out to examine the change of the membrane surface which caused by UV irradiation. The TiO_2 covered RC UF membranes surface are wide variety of TiO_2 nanoparticles (Fig 2b).

In the next series of experiments, the model wastewater was filtered through membranes covered by TiO_2-layers. Analysis of the results obtained by fitting the filtration models (cake filtration, intermediate filtration, standard pore blocking and complete pore blocking) to the experimental data showed that the cake filtration model have given the best correlation.

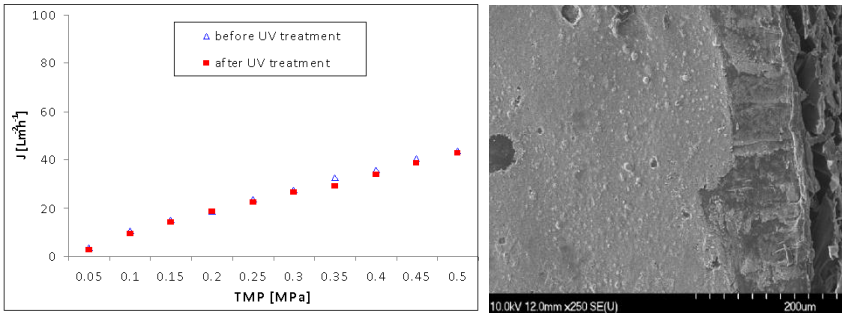

Figure 2: (a) Fluxes with increased TMP, (b) TiO_2 –covered RC membrane surface

From the fitting of Eq. 1 to the experimental data, the initial flux J_0 and fouling coefficient k_c were calculated. It was found, that the initial fluxes were lower in the case of TiO_2 prepared membranes, but the UV irradiation increased it greatly (Table 1). The highest permanent fluxes were achieved with TiO_2 –covered membrane after UV irradiation, which can be explained by the photocatalytic effect of TiO_2. The TiO_2 covering and UV irradiation decreased the fouling coefficient values. The retention of COD similarly changed as the permanent flux. The results also showed (Table 1) that TiO_2-covering increased the retention efficiency without UV light. The 30 min long UV treatment caused flux increasing, therefore the retention values are similar to the retention achieved with conventional RC membrane.

Table 1: Results of calculations

	J_0	J_∞	k_c	R% (COD)
UF	17.9605	5.2287	0.0776	44.85
UF/TiO_2	13.1306	4.5802	0.0108	62.13
UF/TiO_2/UV	17.4708	5.3333	0.0096	42.58

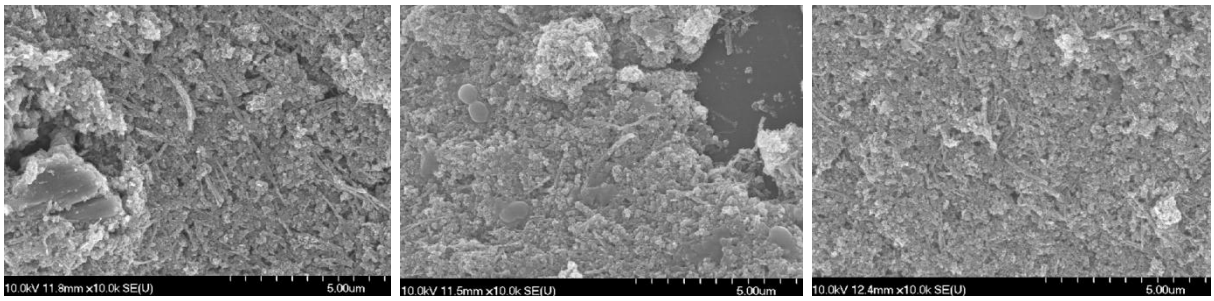

Figure 3: (a) before whey separation, (b) after whey separation, (c) after whey separation and 30 min UV

SEM analysis results showed (Fig 3a/b/c), that whey solution caused gel-layer can be detected on the top of TiO_2-covered membrane surface (Fig. 3b), but after the UV irradiation, the gel-layer on the surface of the membrane could be effectively decreased (Fig 3c).

4. CONCLUSIONS

Following the use of batch stirred cell to compare the regenerated cellulose (RC) membrane and TiO_2 –covered RC membrane filtration efficiency of a model whey containing waste water, analysis of the fall in flux with time indicated that the cake formation model gave the best correlation to the experimental data. However, the UV irradiation damaged the RC membrane, but TiO_2 –covered RC membrane UV irradiation activated the TiO_2 nanotubes, which caused flux increasing and gel-layer formation decreasing, as the non UV irradiated TiO_2-coverd RC membrane, therefore the UV irradiation can be used as cleaning method. Our results showed that the TiO_2 covering slightly decrease the flux which can be increasing by cleaning with photocatalytic method, while the retention of the membrane increasing with TiO_2 covering, and slightly decrease after cleaning.

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APPLIED RESEARCH ON FINE VINAIGRETTE EMULSION PRODUCTION BY SPECIAL MEMBRANE TECHNIQUE

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SUMMARY

In the present work achievement in energy efficient food production and basic product development were targeted. After a series of experiments and data analysis it can be concluded that cross-flow ME is suitable for green production of water-in-oil (W/O) emulsions like vinaigrette, and the technology used looks suitable for industrial applications. Based on experimental data mathematical models were established and process optimization was carried in order out to determine crucial operating parameters and basic sensory analysis test were carried out. Also numerous data were collected for up-scaling purposes.

1. INTRODUCTION

1.1. Vinaigrette

Salad dressing is a sauce for salad usually based on vinaigrette, mayonnaise or another emulsified mixture. Salad dressing was first mentioned by Charles dickens in 1836. Vinaigrette is a mixture of oil and vinegar (plus salt, pepper and other seasonings). The word is from the French *"vinaigre"*, which means vinegar. Vinaigrette has many other names like Italian or French dressing. Vinaigrette should not be added to a salad until just before serving because otherwise the lettuce will wilt (Kipfer, 2011). The ratio of oil and vinegar normally used is 3:1 or 2:1 and vinaigrette is usually manually produced. It is called a temporary emulsion because the oil and vinegar begin to separate as soon as the mixing is stopped. Any kind of oils labeled "vegetable oil" or "salad oil" and also light, neutral-flavored oil like sunflower, canola or soybean oil can be used to make basic vinaigrette. One of the most common variations is to substitute olive oil for salad oil. Extra virgin olive oil is much better than normal olive oil for making vinaigrette. Different kind of vinegar can be used depending on the taste and flavor. Salt, chili and other flavoring agent can be used to change or improve the taste of the final product. Vinaigrette can be classified on the basis of the ingredients, taste and flavor:

- Mustard (and Horseradish) Vinaigrettes
- Herbs and Spices Vinaigrettes
- Vegetable Vinaigrettes
- Citrus Vinaigrettes
- Fruit Vinaigrettes
- Ethnic and Miscellaneous Vinaigrettes
- Oil-Free Vinaigrettes

Health benefits

Balsamic vinegar results in a lower calorie intake compare to other dressings or marinades that are more fattening. Balsamic vinegar also works to suppress the body's appetite and increase the amount of time it takes for the stomach to empty, which can contribute to weight loss by preventing overeating. According to Nutrition Data, balsamic vinegar is a source of calcium, iron, manganese and potassium, which improve the body's functioning and weight loss abilities.

Herb vinaigrette has a deserved reputation for being high in fat and calories, but the calories in this type of dressing deliver nutritional benefits. The vegetable oil in herb vinaigrette has healthy essential fatty acids, and the combination of oil and herbs delivers vitamins and antioxidants. One serving of salad dressing is 2 tablespoons which can contain fat, vitamin E, vitamin K, anti-inflammatory and anti-coronary heart disease agents and it can also help in the reduction of cholesterol intake.

1.2. Membrane Emulsification

Membrane processes have become major tools in the food processing industry over the last 25 years, with the classical reverse osmosis, nanofiltration, ultrafiltration, emulsification and microfiltration processes (Daufin et al., 2001) Membrane emulsification is a new emulsification technique based on the use of microporous membranes (Nakashima et al, 1993). Membrane emulsification was applied mainly to the preparation of O/W emulsions, although a number of studies also reported the preparation of W/O emulsions. (Kandori et al, 1991). The membrane emulsification can be classified in to two main group based on the set-up; stirred cell- and cross flow mode.

Over the last 20 years, there has been a growing interest in membrane emulsification (Joscelyne, Trägårdh et al., 2000; Gijsbertsen-Abrahamse et al., 2004; Charcosset et al., 2004; van der Graaf et al., 2005; Vladisavljevic´, Williams et al., 2005). This method involves using a low pressure to force the dispersed phase to permeate through a membrane into the continuous phase. The distinguishing feature is that the resulting droplet size is controlled primarily by the choice of the membrane and not by the generation of turbulent droplet break-up. The technique is highly attractive given its simplicity, potentially lower energy demands, need for less surfactant and the resulting narrow droplet size distributions. It is applicable to O/W, W/O and multiple emulsions (Vladisavljevic´, Williams et al., 2005). In case of cross-flow membrane emulsification the application of static mixing is considerable to reduce the energy consumption of the operation in large numbers (Koris et al, 2010). According to literature review, discussion of salad dressing or vinaigrette production by ME was not found.

2. MATERIALS AND METHODS

2.1. Optimization of the Emulsification

In the optimization experiments commercial grade sunflower oil was used. The density of the oil was 922 kg m^{-3} and viscosity was η= 69 mPas. Ceramic tube membranes (α alumina) with l.4 µm mean pore size and with $5x10^{-3}$ m^2 active membrane surface were investigated.
In order to increase energy efficiency spiral-ribbon static mixer was installed in the lumen side of the membrane. The length of one complete turn in the spiral was 24 mm, the width was 6 mm and the thickness was 1 mm.

The axial cross-flow velocity in the tube membranes was varied in the range from 0.58 m/s to 1.15 m/s when the recirculated flow-rate (RFR) was set on the pump at 20 L/hr and 40 L/hr, respectively, in order to keep the same shear stress which was obtained by without reducer earlier. In this case, the value of the share-stress was also same as without reducer, namely 9.5 Pa and 19 Pa respectively. The pressure of dispersed phase was assured by air compressor. It was injected from the shell side of the membrane. The oil was re-circulated on the lumen side of the membrane by a common peristaltic pump.

Response surface methodology (RSM) is a collection of mathematical and statistical techniques and it is a good tool for studying the effect of several factors at different level and their influence on each other. To describe the effect of the two operating parameters, driving force (DF) and shear-stress (τ) on disperse phase flux (DPF), droplet size (or diameter) and span, a model based on 3^p type full factorial experimental design was set up. This is a non-linear function relationship where a factor is tested on three levels (minimum, middle point and maximum). The general polynomic regressive function for this model is shown in Eq 1.

$$Y = b_0 + b_L X_L + b_Q X_Q^2 + b_{12} X_1 X_2 , \quad (1)$$

where Y is the dependent variable, b_n symbolizes the constants, X_n stands for the independent variables.

2.2. Preparation of Vinaigrette by ME

Commercial white wine vinegar from white wine 6% was purchased from local market. The 1.5 m/m% water solution was formed as dispersed phase with 15 ml surfactant (Tween 80) and 985 ml vinegar. The "Floriol Mediterrán" oil (sunflower oil 90% and olive oil 10% - from Bunge Zrt.) were purchased from local market as well. The flow rate of oil was set at 40 L/hr and the pressure of vinegar at 2.3 bars.

3. RESULTS SUMMARY

The main results of the 3^p experiments carried out with the membrane with pore size of 1.4 µm it can be concluded that:

1. basic, non-linear working formulas were worked out for vinaigrette production (see Fig. 1);
2. optimal operating conditions were found for vinaigrette production by ME.

Result of pumping power requirement calculations allows concluding that:

3. In general 92% less energy required for recirculation of the continuous phase and emulsion with static mixer (compared to the case when no static mixer was used).

Result of sensory analyses of handmade and membrane produced vinaigrettes:

4. No significant difference between handmade and ME product, however panelists gave little bit higher points to ME vinaigrette.

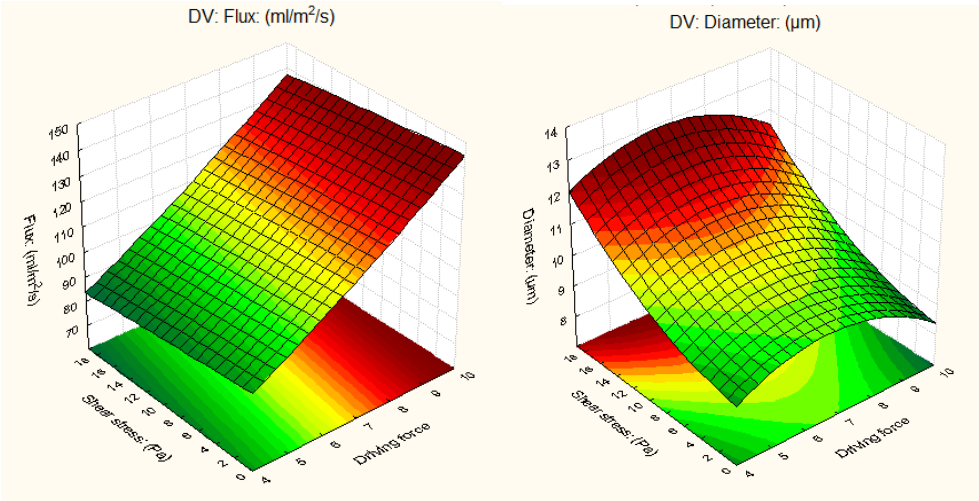

Figure 1: Flux and dropletsize vs. shear-stress and driving force – the surface response models.

Becoming more and more energy efficient is an important action for industrial producers - as well for every single person - to reduce operating cost and reduce impact on the environment. The energy used to process food products is a significant part of the total price as well. In our work it was determined that applying given static mixer inside a tubular ceramic membrane can make the ME process even more energy efficient without any negative effects in terms of productivity and emulsion quality. As a final conclusion, it can be stated that ME (in combination with static mixing) was found comparable with traditional (physical) methods to produce vinaigrette emulsion, due to the method's high productivity and low energy requirement, but also fine emulsion structure is generated in the laboratory experiments and furthermore, the process is friendly to shear-sensitive materials (e.g. proteins).

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CHANGES IN SOME RIPENING CHARACTERISTICS OF SEMI-HARD CHEESE AS AN EFFECT OF HIGH HYDROSTATIC PRESSURE

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SUMMARY

Cost of cheese ripening is quite high, thus acceleration of the ripening process would result in significant savings to the cheese industry. Aim of the study was to investigate the effect of high hydrostatic pressure (HHP; 400, 500 and 600 MPa, 10 min) on some ripening characteristics of semi-hard cheese directly after HHP treatment and during a 4-week ripening period. Aerobic plate counts of HHP treated cheeses were lower by 1.0-1.5 log cycles even at the end of the ripening time compared to control samples. HHP treatment significantly ($p \leq 0,05$) increased the pH of cheese samples, while during ripening pH showed a decreasing tendency. Lightness values of pressurized samples were lower, whilst green and yellow hue values were higher than those of control cheeses. Firmness, elasticity and chewiness of samples decreased, but cohesivity increased during ripening. Organoleptic analysis indicated that HHP treated cheese samples were more preferred than control ones both directly after treatment and at the end of the ripening period. HHP treatment induced changes in the ripening characteristics that developed only after 1-2 weeks of ripening in the control cheese.

1. INTRODUCTION

Modern consumers tend to choose food products which are minimally processed, high in nutritional value, safe and good tasting at the same time, hence food industry is continuously aiming to develop novel food processing technologies. One of these technologies is high hydrostatic pressure (HHP) treatment, which enables destruction of microorganisms without causing significant changes to the colour, flavour and nutritional attributes of the food. In addition, high pressure can cause rheological changes in food which result in beneficial sensory and structural effects (Datta and Deeth, 2002).

The ripening of cheese is a slow and expensive process and cannot be completely controlled. Costs of cheese ripening are rather high, thus the development of an efficient way to reduce the ageing time would result in significant savings to the cheese industry (El Soda, 2002). The principal methods used for this purpose are elevated temperatures, addition of exogenous enzymes or cheese slurries, and the use of proper starters or adjunct cultures (San Martín-González et al., 2006). As an alternative method, high pressure treatment could be used instead for acceleration of cheese-ripening. Diverse research papers have reported that application of high pressure to cheese could accelerate ripening, in particular proteolysis in cheese (Kolakowski et al., 1998; Messens et al., 2000, 2001) however, others reported that the influence of high pressure depended on the treatment applied and, in some cases, there were no effects on the ripening process at all (Wick et al., 2004; Rynne et al., 2008).

There have been several reports on the effects of HHP treatment on acceleration of proteolysis and flavour development in different cheese varieties, including Cheddar (Yokoyama et al., 1992; O'Reilly et al., 2000) Gouda (Messens et al.,1999) Camembert (Kolakowski et al., 1998), and goats cheese (Saldo et al., 2002).

Majority of the research conducted so far in this topic dealt with the effect of HHP on the proteolysis of cheese, not focusing on the changes in those characteristics, which significantly determine the consumers' decision, namely rheological and sensory properties induced by the treatment. The aim of the current study was to examine the effect of HHP treatment (400 MPa, 500 MPa and 600 MPa, 10 min) at different stages of ripening on color, texture and organoleptic properties of a semi-hard artisanal cheese.

2. MATERIALS AND METHODS

2.1. Cheese samples and HHP treatment

Fresh semi-hard artisanal cheese (fat content in dry matter 45%) was used in the experiments. Five cheese bricks were cut in six blocks each, thus providing 30 cheese samples with approx. 300-400 g weight. Samples were packed into vacuum pouches (Cryovac BB4L foil bags; Sealed Air Corporation, USA) and vacuum sealed (Multivac A300/16, Multivac, Germany).

Fifteen cheese samples were randomly chosen and pressurized (FPU-100-2 000; Resato International B.V., the Netherlands) at 400, 500 and 600 MPa, respectively, for 10 mins. The rest of the cheese blocks were control samples (no treatment). Control and pressurized samples alike were ripened at 14 °C for 4 weeks. Samples were taken directly after HHP treatment (0. week) then every week during the ripening period.

2.2. Aerobic plate count

Aerobic plate counts were determined as follows: 10 g of cheese sample was placed in a stomacher bag (with nylon mesh bag, pore size 1 mm) with 40 cm^3 of diluents (1 g peptone, 8.5 g NaCl in 1000 cm^3 distilled water). Five-time dilution cheese homogenates were stomached for 2 minutes (Stomacher Bag Mixer, Interscience, France), and 10-fold dilution series were prepared. These were routinely cultivated on Plate Count Agar (PCA, MERCK KGaA, Germany). PCA plates were incubated at 30 °C for 2–3 days. Measurements were performed in duplicates.

2.3. Determination of pH and solids content

Cheese samples were analyzed in triplicate for pH (Testo 206 pH-meter, Testo AG, Germany) and solids content (Kern MLS, Kern & Sohn GmbH, Germany).

2.4. Color measurement

Color measurements were carried out using a Minolta CR-200 colorimeter (Konica Minolta Holding, Japan). Color of the cheese surfaces was described using the CIELAB scale: L* (lightness); a* (green to red hue) and b* (blue to yellow hue). Measurements were performed five times per sample on different points on the cheese surface. Total color differences (ΔE*) were computed using the following equation:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \qquad (1)$$

2.5. Texture profile analysis

Cylindrical samples of 12 mm height and 12 mm diameter equilibrated at 14 °C were analyzed by means of a SMS TA-XT2 Texture Analyzer (Stable Micro Systems Ltd., UK). The vertical displacement speed and the deformation were set at 120 $mm*min^{-1}$ and 70%, respectively. Six replicates per each sample were analyzed.

2.6. Organoleptic analysis

Organoleptic analyses were carried out by 10 assessors directly following HHP treatment and then weekly during ripening. Sensory descriptors of the cheeses were as follows: color,

odor, flavor, texture (hardness, elasticity, adhesiveness and crumbliness), and overall acceptability. Assessors marked responses on a 10-point intensity scale.

2.7. Statistical analysis

Statistical analysis of data was performed by analysis of variance (ANOVA) and relevant post-hoc tests (LSD and Tukey pairwise comparison) using SPSS Statistics version 18. Statistically significant effects were defined at the 95% significance level.

2. RESULTS

3.1. Aerobic plate count

Aerobic plate count of cheese samples was determined at the end of the ripening period. Control sample and the one treated by 400 MPa had very similar plate counts (~9 log CFU/g), while 500 or 600 MPa pressure decreased the microbial load of the other samples by 1.0-1.5 log cycles.

3.2. pH and solids content

Directly after HHP treatment, pH of cheese samples significantly ($p \leq 0,05$) increased, by 0,4-0,5 units on the average, but during ripening pH showed a decreasing tendency. Cheese samples pressurized at 400 MPa had the highest rate of acidification and the control the lowest one, although by the end of the 4th week their final pH was very close to each other. The other two samples had higher and almost identical pH values. Solids content of cheese samples varied between 52-58 g/100 g and didn't change significantly during ripening mainly owing to the vacuum packaging.

3.3. Color measurement

Lightness value of HHP treated samples was lower than that of the control samples directly after the treatment. During ripening, all the cheese samples became darker. At the end of ripening the control sample had the lowest L* value, while the pressurized samples showed significantly higher L* values. The biggest difference in a* was observed in case of the samples treated by 600 MPa, a slightly stronger greenish hue developed over time. Yellow hue (b* value) of the cheese samples slowly but gradually increased during ripening, but the differences between control and pressurized samples were not significant. Highest color differences (ΔE^*) were calculated between control and by 600 MPa treated samples from the first week on.

3.4. Texture profile analysis

Firmness, elasticity and chewiness of samples decreased, but cohesivity increased during ripening. Firmness of by 500 and 600 MPa treated samples steadily and gradually decreased having very similar firmness values, while firmness of control and at 400 MPa pressurized samples decreased abruptly by the end of the first week and remained almost constant during the studied period. However, after the 4-week ripening time, the differences in firmness diminished. Elasticity decreased parallel with the softening of cheese texture. After HHP treatment, no significant differences could be detected between the samples, but after 4 weeks, elasticity of control and at 400 MPa treated samples was lower than that of the

samples treated at higher pressures. High pressure increased cohesivity, 500 and 600 MPa pressures resulted in more cohesive texture. Chewiness was getting lower during ripening, but in case of samples treated at higher pressures, a sudden decrease was detected after the 2nd week, while control and 400 MPa samples reached their "final" chewiness already during the 1st week and remained constant since then.

3.5. Organoleptic analysis

Assessors considered HHP treated samples more firm and less crumbly than the control sample. They found no pronounced differences in color, smell and elasticity between the samples. The overall acceptance of samples treated by 500 and 600 MPa, respectively, was the best in every occasion. The assessors deemed less and less differences from week to week between the tested characteristics of control and pressurized samples.

4. CONCLUSIONS

High pressure induced marked changes in ripening characteristics of cheese, although not all of those were similar to changes developing during ripening. From the applied pressure levels, with the use of 400 MPa treatment the ripening time may be reduced by 1-2 weeks.

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STRATEGIC DILEMMAS OF HUNGARIAN FOOD ECONOMY IN GLOBALISING WORLD

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SUMMARY

The article analyses four strategic problems of development of Hungarian agriculture and food industry. It is an inherent contradiction all over Europe, that the agricultural production is much more fragmented, than the food processing, and the later is relatively de-concnetrated compared to the highly concentrated food trade. A possible solution of this problem is not the creation of large-scale agricultural enterprises, but the promotion of co-operatives of small-and medium size farmers. Without these there seems no possibility to achieve an even-quality, balanced supply of agricultural products. Utilisation of agricultural row materials for energy production is a highly controversial question in European agricultural and energy policy. Hungary could considerably increase its bio-based energy production, but this capital-intensive development necessitates a highly predictable economic environment. The productivity of Hungarian food industry is in the mid-field of Europe. This can be explained by considerable role of multinational companies. Increasing share of small-and medium size enterprises will decrease this efficiency, but another considerations, first of all workplace creation should be taken into consideration, too. The export structure of Hungarian food economy is dominated by row materials. Relative price level, achieved in Hungarian export is far lower, than that of the most important competitors. Under these conditions there is an increasing importance of export oriented economic policy. This should be based on (1) market diversification, (2) concentration of supply, (3) innovation of products, based on clusters of producers and R+D organisations.

AGRICULTURAL SUPPLY CHAIN DEVELOPMENT

It is a generally accepted fact, that the stability and predictability of agricultural raw materials is a necessary precondition of food industrial development. Relative scattered structure of agricultural production and the concentrated food industry is a natural phenomenon in Europe. According to the estimation of Grievink (2003) there are approximately 110 buying desks in European retail system, which buy the overwhelming majority of agricultural and food industrial products. At the same time, approximately three quarter of agricultural production is produced by about 3 million agricultural family farms, but this contradiction is counterbalanced by strong cooperative-movements. In Hungary, the cooperative market share is well below the EU-average: in case of dairy products 31% (EU average: 57%), pigmeat 25% (EU average: 27%), fruits and vegetables: 18% (EU: 42%), cereals: 12% (EU: 34%). Under these conditions there seems to be three ways of development: (1) further concentration of agriculture, achieving a relatively high level of efficiency (especially in case of arable-land products), but loosing its potential to create workplaces; (2) development of agro-franchise systems, based on so-called integrated agriculture. This seems to be a workable construction under current circumstances, but the integrator organisations are reluctant to finance the more risky agricultural activities, they prefer the predictable, rather simple product-paths; (3) development of cooperative –based agricultural organisation. This later way is in line with results of latest EU-wide survey, which highlights, that in regions with a larger market share of cooperatives, the prices of agricultural raw materials (e.g. milk) are higher and the financial position of agricultural producers is more favourable, especially from point of view of liquidity. Our on-site research highlight the importance of strengthening the cooperative movement especially in sphere of horticultural products, because there is a continuous efforts of import liberalisation in EU, and the relatively cheap products from the developing world as well as the strengthening EU-Turkey foreign trade relations will further increase the competition in European market.

Based on our on-site direct-question survey the most important problems in organisation of cooperatives are as follows: (1) unreliable institutional context; (2) considerable share of grey and black market; (3) lack of positive patterns (4) too costly cooperative membership for small farmers; (5) relative low level of mutual trust; (6) under-developed financial infrastructure. It is a very important advantage of cooperative, that they can offer a safe and fast payment for their members, but this necessitates the sufficient level of working capital as well as revolving funds.

PRODUCTION OF RENEWABLE ENERGY FROM AGRICULTURAL PRODUCTS

In last decades there has been an intense R+D activity as well as considerable investment with purpose of increasing the share of renewable energy resource development. There has been a rapid development in European renewable energy programs (Charles, 2013).
It should be highlighted, that the bioethanol production is an extremely capital-intensive investment, and the rate of return is relatively low, even in case of plants, offering a relatively high profitability (Fig. 1).

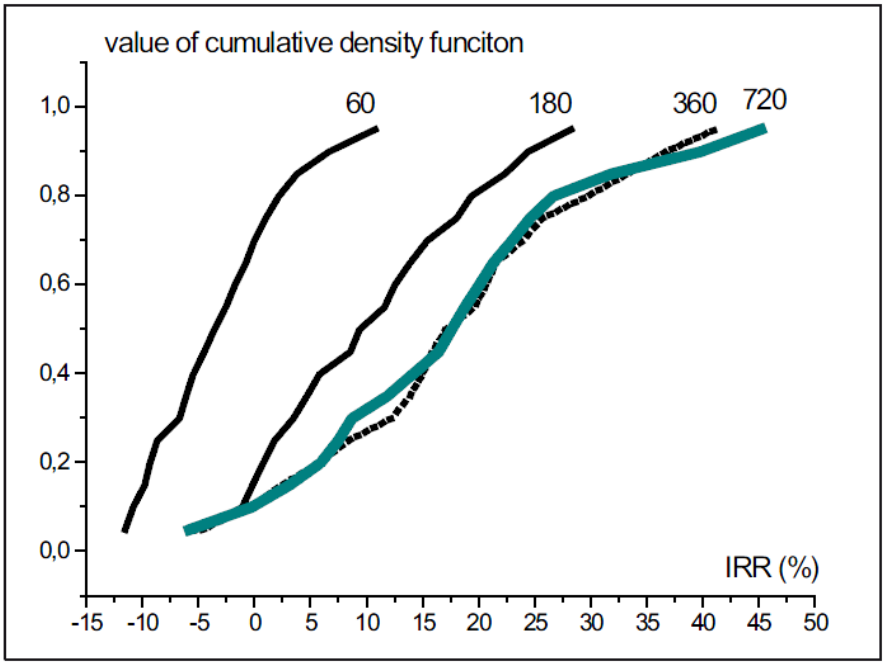

Fig.1: Expected profitability (rate of return) of bioethanol-plants with different production capacity (m^3/day)
Source: own calculations

The latest measures of Hungarian government have undermined the economic bases of bioethanol-production. These negative effects of changes in regulatory framework considerably decreased the selling of bioethanol (Table 1)

Table 1: Glamour and gloom of bioethanol program in Hungary

Years	E85 turnover (million l)	Excise duty (Ft/l)	VAT (Ft/l)	Income from excise duty (million UF)	Income from VAT (million HUF)	Total income from taxes (million HUF)
2010	36,2	19,2	48	697	1738	2435
2011	39,2	23,9	63	939	2470	3409
2012	3,6	77,5	72	284	259	543

Source: http://e85power.hu, calculations based on official data of Ministry of National Economy

PERFORMANCE AND EFFICIENCY OF FOOD INDUSTRY

Comparing the efficiency of Hungarian food industry to another states based on output/worker indicator offers a favourable possibility to compare of different European food industrial systems. Applying this analysis on base of statistical data of Food and Drink Europe there seems to be a considerable backwardness. However, this comparison is misleading, because it should be taken into consideration the differences in purchasing power of national currencies. After this correction we obtain a much more realistic picture on efficiency of Hungarian food industry. According to this comparison, the Hungarian food industry is in the European mid-field position. In latest Hungarian documents there is a high emphasis on importance of support of micro, small and medium-size enterprises. These measures necessarily will decrease the efficiency of food production, but will be able to increase the role of food industry in workplace-creation. A series of case studies have proven, that support of investment of SMEs is not enough: they need a relatively easy access to revolving capital credit as well as some additional "adjuvant" measures of industrial policy. The most important of these are as follows: (1) upgrading of technological as well as management and marketing knowledge and skills; (2) improvement of access to consumers: development of direct channels of marketing; (3) support of innovation, quality assurance and another functions (e.g. vocational training, short-term professional courses) by promotion of organisation of clusters. .

MARKET-STRUCTURE OF HUNGARIAN FOOD ECONOMY

Traditionally, the Hungarian agriculture and food production have been export-oriented. Share of agro-food products in Hungarian export has been ca. 60 % in 1920 and ca. 22% in 1980. According to some estimations, the Hungarian agro-ecological potential is able to fulfil the demand of more than 15 million people. The program of current Hungarian government "The Programme of National Cooperation" has determined a paradigm-shift, laying a much more emphasise on utilisation of possibilities of domestic market, determining, that "the important markets for Hungarian agriculture are the domestic and local markets." The basic pillar of this approach of development is the increasing importance of domestic purchasing power. However, under conditions of global economic crisis and instability it is rather difficult to build the development on domestic market, especially in such period, when there is a general increasing of world market prices of agricultural and food industrial products. Under these conditions there is an extremely high necessity of utilisation of foreign market possibilities.

The performance of Hungarian food economy in foreign markets has been analysed by two methods: analysis of product-portfolio and by analysis of price level, compared to the most important European competitors. The database of analysis has been the statistical data-collection of FAO, the so-called faostat data. Based on this it has become evident, that in last years ca. 80 % of Hungarian agro-food export has been raw materials. The concentration of

Hungarian export is much more higher than that of another European countries. To determine the price-level, we have identified the most important product-categories of Hungarian agro-food export: There are 95 products/product categories of which the share in agro –food export is higher than 0,1%, considering the 2005-2010 period. These products cover ca. 90% of value of Hungarian foreign trade in export of agricultural and food industrial products. In this calculation we have not taken into consideration the value of export of living animals. In next phase of our research we have determined the relative price level of Hungarian export products to average export prices of the most important five exporter in given product group.
In general it can be determined, that the price-level of Hungarian products is far below that of the most important competitors. There are just a few products, in case of which a considerable export-price advantage could be achieved. In case of some arable –land products there are just two products, where the Hungarian export prices are higher than the prices of competitors.
These are the dry beans (298%) and dry peas (238%).
It is very important to seen, that there is not a single product in Hungarian fruit production, where the Hungarian export would be capable to achieve an above-average price! In case of vegetable products the situation is a bit better. In case of cabbages (115%), lettuce (173%) and peppers (111%), mushrooms (104%) the prices are relatively favourable.
In case of processed plant products there is a price-advantage in case of soybeans cake (114%), malt (106%), margarine (111%), paste of tomatoes (109%),refined sugar (107%), and dehydrated vegetables (113%)
In export of animal products the price level of eggs (241%), chicken meat (139%), canned meat of chicken (104%), sausages (136%), pig meat (120%), rabbit meat (108%), turkey meat (145%) are above the level of competitors.
Among the relatively "high price" products there are two categories, which are not produced in Hungary, just the storage, packaging and logistical distribution takes place in Hungary, that's why these products appear in Hungarian export, but practically here we see just a re-export activity: this is a case in export of bananas ((111%), and tea (143%).
As a summary it can be stated, that the export-price level of Hungarian agricultural and food industrial products in most cases are well below the price level, achieved by the most important exporters of the given product category. According to our interviews with the leading experts of Hungarian agriculture, food industry and trade one of the most important cause of this situation is the scattered and often chaotic structure and organisation of Hungarian foreign trade: under these conditions there is just a limited possibility to achieve a higher price level: the different export organisations often compete against each other by low prices.

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EXTRACTS FROM THE RESEARCH OF THE NATIONAL COLLECTION OF AGRICULTURAL AND INDUSTRIAL MICROORGANISMS

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SUMMARY
The National Collection of Agricultural and Industrial Microorganisms (NCAIM) was founded in 1985 and acquired the status of an International Depositary Authority in 1986 and since then it has been accepting patent strains under the regulation of the Budapest Treaty. The NCAIM takes an important role in the ex-situ conservation of microbial biodiversity, and internationally recognized research activity is also performed in the Collection. Important fields of our research include the investigation of the biodiversity and the systematics of yeasts. In this contribution some substantial results of our research are briefly presented.

1. INTRODUCTION

The application of sequence analysis and the parallel availability of a large, freely accessible database of nucleic acid sequences (e.g. GenBank, http://www.ncbi.nlm.nih.gov) have provided an excellent means for exploring the biodiversity of microorganisms, including yeasts. As a result, the number of described yeast species has dramatically increased during the last decades (Fig. 1). The D1/D2 LSU rRNA gene sequences were determined and made publicly available practically for all known yeast species (Kurtzman and Robnett, 1998; Fell et al, 2000), and since then, the deposit of these sequences in public databases is a prerequisite of describing new yeasts species. Therefore in case of yeasts the D1/D2 LSU rRNA gene sequences have also become an irreplaceable tool for rapid and accurate identification. The National Collection of Agricultural and Industrial Microorganisms (NCAIM) takes an important role in the *ex-situ* conservation of microbial biodiversity, and is a workshop of the investigation of yeast biodiversity in Hungary, both in natural and in man-made environments. The research activity of the NCAIM is exemplified by some results achieved in the field of yeast systematics and the exploration of yeast biodiversity in the last two decades.

2. *YARROWIA LIPOLYTICA* AND RELATED SPECIES

Yarrowia lipolytica is a common food spoilage organism (Deák, 2008), however, it has several properties, including the production of lipase and organic acids, which have been exploited by the industry (Coelho et al, 2010). *Y. lipolytica* has been the only known member of the genus for a long time, and was considered to be able to produce urease enzyme, which is not produced by other members of the class Saccharomycetes (Kurtzman and Fell, 1998). Our studies revealed that *Y. lipolytica* was actually unable to produce urease and the colour change of the indicator in the widely used Christensen's urea agar was caused by a non-specific alkalization reaction instead of urease activity (Péter & Deák, 1991). Later, several additional undescribed species of the *Yarrowia* clade were recovered from different meets (pork, beef and poultry). *Candida galli* was already proposed as a new species (Péter et al, 2004), and currently three additional *Yarrowia* species are being described.

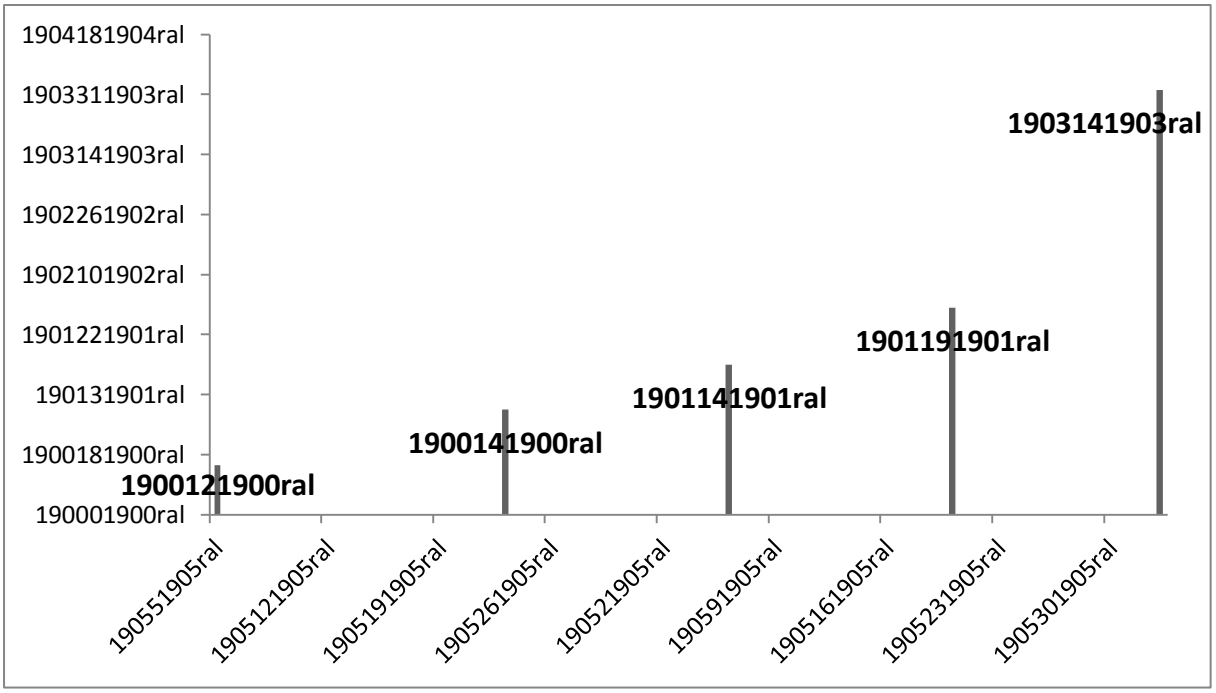

Figure 1: The number of the yeast species treated by the subsequent editions of "The Yeasts, A Taxonomic Study"

3. THE FAMILY TRICHOMONASCACEAE

The family Trichomonascaceae mostly comprises yeast genera characterized by the formation of septate hyphae. Currently, one non-hyphal genus, Wickerhamiella, is also assigned to this family, however, its assignment is tentative, because of the weak support of the basal lineages of the DNA sequence based phylogenetic trees (Kurtzman, 2011). Our investigations revealed that rotten wood is a suitable substrate for isolation of hyphal members of the family Trichomonascaceae. During the last two decades we have isolated, mainly from rotten wood samples, numerous filament-forming yeast strains belonging to this family. Sequence based identification of these isolates has revealed that several strains represent undescribed species and even undescribed genera, which can be recognized only from gene sequence analysis. In addition to four new species, until now, two novel genera, *Spencermartinsiella* and *Diddensiella* were proposed (Péter et al, 2011a, 2012).

4. *SACCHAROMYCES* ON GRAPES

The spontaneous fermentation of grape must is generally initiated by non-*Saccharomyces* yeasts, but after two or three days, more strongly fermenting and more ethanol tolerating species of *Saccharomyces* take over the fermentation (Fleet & Heard, 1993). The number of *Saccharomyces* cells on grape berries is rather low, and the role of *Saccharomyces* strains inhabiting the grapes in spontaneous fermentation has been debated (e.g. Martini, 1993; Mortimer & Polsinelli, 1999). The isolation of *Saccharomyces* strains from grape may be necessary during studies of the ecology of the winemaking, and grape-derived *Saccharomyces* strains may also be considered as potential starters. Due to their low number, it is difficult to isolate *Saccharomyces* strains from grapes without enrichment. The most common method for *Saccharomyces* isolation from grapes is crashing and fermenting the grapes under laboratory conditions, followed by serial dilution and plating on a suitable agar medium (Martini, 1993). In order to provide a simple and effective alternative method for the isolation of

Saccharomyces strains from grape, we applied enrichment in a medium supplemented with 10% (v/v) methanol. The method, although not fully selective, proved to be a suitable alternative for isolating *Saccharomyces* strains from grape. Sixteen of the 18 grape samples yielded *Saccharomyces* strain(s), and more than 70% of the isolates belonged to the genus *Saccharomyces* (Péter et al 2011b).

5. METHYLOTROPHIC YEASTS

Methylotrophic yeasts can grow with methanol as a sole carbon and energy source. Some of them are utilised by biotechnology as producers of heterologous proteins. As with the wider yeast domain, the number of known methanol assimilating yeasts is growing rapidly. While in 1998 only 29 methylotrophic yeast species were known (Kurtzman and Fell, 1998) their number currently is approaching 80. The vast majority of them belong to the ascomycetous yeasts and until recently the teleomorphic species were classified in the genus *Pichia* (including the former genus *Hansenula*), while most anamorphic species are assigned to the genus *Candida*. Based on the results of the analyses of nucleic acid sequences, some of the teleomorphic methanol-assimilating yeast species were proposed to be reclassified in three genera: *Ogataea*, *Kuraishia* and *Komagataella* (Yamada et al, 1994, 1995). These proposals have been gradually accepted and additional species were transferred to the newly established genera and the novel species were also classified in these genera (Kurtzman et al, 2011).

During our study of the methanol-assimilating yeasts, we established that *Komagataella* (*Pichia*) *pastoris* is the predominant yeast species in different tree exudates and in rotten wood, in Hungary, and a closely related species *K.* (*Pichia*) *pseudopastoris* was described based on isolates recovered from rotten wood. With the application of a selective isolation procedure we revealed that plant leaves, known to harbour mainly basidiomycetous yeasts, are inhabited also by ascomycetous methanol-assimilating yeast species, although they occur in low cell densities. Different methanol-assimilating yeasts were regularly isolated from leaves, including the above-noted *K. pastoris* and two earlier unknown yeast species, *Ogataea allantospora*, a species forming allantoid ascospores and *O. nitratoaversa*, a species unable to assimilate nitrate as a sole nitrogen source. As until the observation of the ascospores of *O. allantospora*, no *Ogataea* species were known to produce allantoid ascospores and the diagnosis of the genus stated that “potassium nitrate is assimilated”, emendations of the genus *Ogataea* were proposed to allow the inclusion of species that form allantoid ascospores and species which do not assimilate nitrate as a sole nitrogen source (Péter et al., 2007, 2008). Altogether, nineteen methanol-assimilating yeast species were described with the contribution of NCAIM researchers.

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BIOTECHNOLOGY, BIOSAFETY AND DIVERSITY OF YEASTS *SACCHAROMYCES CEREVISIAE*: CAN ALL THIS BE MANAGED?[1]

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Professor Emeritus Dr. Tibor Deák, microbiologist from Budapest, awarded by honorary doctorate from his Alma Mater, the University of Szeged, by Pro Facultate award from the University of Food Technology and Horticulture, by a Széchenyi Professorial Fellowship, and with the Knight Cross of the Republic of Hungary, contributed to the area of food microbiology over 350 peer-reviewed publications, books, and chapters, beside teaching in Hungary and abroad. Professor Deák was an outstanding educator and internationally renowned food microbiologist, particularly as a specialist in yeast taxonomy, identification, and ecology. He earned his Ph.D. on The Microbiology of Lactic Acid Fermentation at Eötvös Loránd University in Budapest in 1963. In 1967, he started at Department of Food Technology at the University of Food Technology and Horticulture in Budapest (currently Corvinus University of Budapest) where he worked 46 years. In 1974, he became the head of the new Department of Microbiology and Biotechnology, later Dean of the School of Food Technology at the University of Food Technology and Horticulture, and finally its chancellor. He established National Collection of Industrial and Agricultural Microorganisms. Within ICFMH, he was Vice President and one of the establishers of the International Journal of Food Microbiology (IJFM) and very active person until 2010. He was also an editor or on the editorial board of three international journals, including the IJFM, as well as a Commissioner of the ICFMH (Anon 2013), the International Commission on Yeasts and a member of the International Commission on Food Mycology. He organized ICFMH Food Micro Symposium in Budapest in 1983 entitled "*Associations and Interactions*", and ISSY in 2003 entitled "Interactions between yeasts and other organisms". For that reason I feel that an overview on yeast *Saccharomyces* would complement our respect to Professor Emeritus Dr. Tibor Deák.

In our 20 years long lasting cooperation with professor Deák, personally and with his co-workers, we exchanged many students and teachers in research, but also on pedagogical programmes (Raspor et al., 2012; Maraz, 2012). The results were presented at national and international meetings (Raspor et al., 1997,1998; Smole Možina et al., 1998) and published in relevant journals (Smole Možina et al., 1997; Maraz et al., 2003). A domesticated yeast from the species *Saccharomyces cerevisiae* has been known as traditional industrial yeast but also recognized as a new production vehicle in modern biotechnological processes. The organism as it is present in traditional fermentation like wine fermentation is the closest to wild strain if compared to traditional brewing industry or bread industry, not mentioning pharmacy industries which also use this organism for various purposes. This organism has many potent and permanent contacts with humans via food and health products what makes it important in terms of biodiversity, biotechnology but also biosafety. During last century it has been shown that this particular yeast S. *cerevisiae* is a good artisan, industrial and nutritional actor, but

[1] *This lecture is devoted to Professor Emeritus Dr. Tibor Deák, for his achievements in the area of yeast research*

also an organism which might compromise health of humans or can also be used for improvement of safety, e.g. for removal of mycotoxins or fungicides (Bizaj et al., 2009; 2011). As a good model organism for biology, biochemistry and nutrition, it opened the discussion about its communication abilities in its intimate surroundings. Analyzing yeast *S. cerevisiae* regarding food production and spoilage, nutrition, health and pathogenicity we had demonstrated its activities in this respect as we studied it in the last years. We have been able to show a huge biodiversity of *S. cerevisiae* strains during wine fermentations but also some mechanisms how to control them (Povhe Jemec et al., 2005). *S. cerevisiae* was found as a good model organism for elucidation of metals' metabolism in higher eukaryotes. We studied yeast stress response to Cr(VI) (Jamnik and Raspor, 2005), accumulation of different minerals (Zn, Cr, Fe), transport mechanisms, mineral localization and storage in yeast cells (Paš et al., 2007). We showed that *S. cerevisiae* in the stationary phase is an appropriate eukaryotic model to study changes in cell proteome, oxidation status and energy metabolic activity in response to different bioactive compounds (Jamnik et al., 2007; Zakrajšek et al., 2011; Petelinc et al., 2011). On the other hand, the pathogenic nature of *S. cerevisiae*, which place it as an emerging opportunistic pathogen, challenged us to develop new methodological approaches for identification of virulent features among isolates from human milieu (Zupan and Raspor, 2008; Zupan et al. 2010). In accordance with the focus of our studies in last decade we still see yeast as an important organism. However, it is more and more clear that it has many faces and can be recognized as a driving force in many processes, but sometimes also an organism which can compromise our health, products or services. Development of yeast *S. cerevisiae* picture is getting extremely heterogeneous. Fast developing tools in molecular biology and biotechnology are giving us new possibilities for research, but also new opportunities for yeast applications in further practice. Following this challenges we introduce a method for monitoring *S. cerevisiae* quorum sensing signalling (Zupan et al., 2013) and currently, we are exploring interactions between cells and other yeast species during wine fermentations to better understand mechanisms, which make *S. cerevisae* the most important eukaryotic industrial microorganism.

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FOOD PHYSICS IN THE FIELD OF COCOA AND CHOCOLATE

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SUMMARY

Physical properties and laws as well as their proper application to praxis are important for the production of foods with high quality in an economical way. In this paper the importance of Food Physics on different examples in the food chain from cocoa to chocolate and a confectionery bar will be shown. Starting with harvest, colour and texture of the cocoa pod could be useful to determine the ripeness. The degree of fermentation can be determined by the cut test. It will be reported about the drying behaviour of Indonesian cocoa and the influence of drying on the aroma precursors glucose and fructose. For a wafer chocolate product the special cutting and sound design of the wafer and the flow behaviour of chocolate for enrobing will be presented. Finally reasons for fat bloom on chocolate and chocolate products and possibilities for prevention are told.

1. INTRODUCTION

Quality defects of cocoa and chocolate and chocolate products are often caused by improper processing methods. In the case of cocoa beans (e.g. too early harvest, no proper fermentation, drying and storage) this can result in a total loss or a discount of price, in the case of chocolate and chocolate products (e.g. with fat bloom) that product cannot be sold or even has to be taken out of the market. Therefore it is necessary that the physical properties and laws are known well and properly applied to the processing methods. Beside fermentation of cocoa, where the aroma precursors like reducing sugars and amino acids are formed which are converted into the final aroma compounds during the roasting process, drying of cocoa beans is the most important process step influencing raw cocoa quality. The drying process is needed to stop fermentation and prevent overfermentation with appearing off-flavours and to reduce the initial moisture content of 50 - 60 % after fermentation to 7% to make the cocoa beans storable. This paper reports about experiments with a drying unit in Jember/Indonesia on the influence of drying parameters on the kinetics of reduction of glucose and fructose in cocoa beans during drying.

For a wafer chocolate product the texture of the wafer is very important for the overall preference of the consumer. Mostly the consumer expects that the wafer is dry, hard and brittle and breaks with a nice sound. The way to fulfill this desire is shown roughly. Cutting of such a hard and brittle wafer is not easy. The solution will be presented.

To produce a filled chocolate product we mostly enrobe the center (e.g. filled wafer block) with chocolate. For a correct enrobing the chocolate has to have the proper flow properties.

Fat bloom on chocolate or chocolate products is a disastrous thing. It is not unhealthy, as it is just fat, but the consumer thinks that mould is on the surface due to the white to grey layer and he will complain about the product and probably will not buy that product a second time. So strong efforts have to be undertaken to prevent this serious form of a quality defect. In this paper reasons for fat bloom and possibilities for prevention are shown.

2. MATERIAL AND METHODS

The research on the drying behavior of cocoa and the influence of the drying conditions on the kinetics of the reduction of the aroma precursors glucose and fructose have been conducted at the Indonesian Coffee and Cocoa Research Institute (ICCRI) in Jember, East Java. The cocoa used for the tests was "bulk cocoa", a Forastero hybride, which is the most widespread cocoa in Indonesia. The cocoa beans had been partially depulped (depulping rate of 30 %) by a mechanical drum depulper to improve fermentation and then fermented in a 2-stages process (500 kg fresh beans, displacement to a second box after 48 hours and mixing) with a total fermentation time of 120 hours. The fermented cocoa beans were subsequently dried in a thin layer batch dryer. Temperature was varied between 30 and 80 °C. But the trial at 30 °C was not further evaluated, as mould occurred during the first hours of drying. In addition 2-stages drying regimes were evaluated. The reducing sugars and the acetic acid in the cocoa beans were determined by enzymatic analysis. All concentrations of reducing sugars and acetic acid are relating to dry base (d.b.), moisture content is relating to wet base (w.b.).

3. RESULTS

3.1 Drying behaviour and kinetics of aroma precursor reduction during drying of Indonesian cocoa beans

Drying time to a moisture content of 7 % ranged from 70 hours at 40 °C drying temperature to 8 hours at 80 °C (air velocity of 0.2 m/s). By the partially depulping before fermentation the drying time at 40 °C could be reduced from 100 hours to 70 hours which is a great advantage. The results show a strong influence of drying temperature on the reduction of the aroma precursors glucose and fructose. Particularly glucose is affected significantly at high temperatures, when moisture content is below 25 %. At 80 °C it is reduced by 95 %, at 40 °C by 10 %. Fructose is more stable, maximum reduction reaches 20 % at 80 °C. A maximum concentration of glucose and fructose in raw cocoa is achieved to be available at the following roasting process, when drying temperatures do not exceed 40 °C after reaching a moisture content of 25 % w.b. The conditions at the beginning of the drying process are not influencing the content of the reducing sugars, as long as moisture content is above 35 %. Below this moisture content the influence of drying temperature on the reduction of glucose and fructose increases obviously. It can be assumed that the decrease of the reducing sugars during drying is caused by the formation of Amadori compounds. The assessment of that formation from the point of flavour development needs further investigation. Beside the importance of reducing sugars and amino acids for flavour development, also the acetic acid content in the cocoa beans plays a role. Low drying temperatures reduce the acetic acid content significantly. This effect can be mainly attributed to the long drying time, which gives acetic acid the opportunity to evaporate. With increasing temperature the acetic acid content increases as well. At a drying temperature of 80 °C acetic acid content is almost as high as after fermentation, which means that there is no acetic acid reduced during this short drying period. So time seems to be the more important parameter. Another reason could be the hardening of pulp residuals at higher drying temperatures, which prevent that volatile acids pass through the shell of the cocoa bean.

3.2 Wafer chocolate product: Sound design, cutting, enrobing, fat bloom

Marketing experts found out that in the case of fine baked goods (wafers, biscuits, cookies), often coated with chocolate, mostly the consumer expects not only a nice eye appeal of the product but also a nice sound for the ear when the product is eaten. Therefore a new discipline in the sector of product design came up: Sound Design. In the meantime at least all big companies include this in their developments. If the sound spectrum is between 7000 and 12000 Hz during the bite procedure the product is extreme to very crispy, which is preferred especially by younger people. Older people usually prefer deeper sounds.

Cutting of such a hard and brittle wafer is not easy. Cutting with (rotating) knifes is not a good solution. Much better is the performance by cutting with thin (0.3 – 0.6 mm) cutting wires located in a frame. The force required for the cutting procedure is lower in this case as friction at the cutting sides is much less and material displacement is minimal which leads to a better quality of the cut surfaces and less loss of material.

For a correct enrobing of wafer blocks with chocolate the chocolate has to have the proper flow properties. In a praxis test it can easily be shown that for the enrobing procedure the yield value is the relevant physical property. In an example where the Casson viscosity is in both cases 3.5 Pa s, but the yield value of the first sample 3.5 Pa and that of the second 16.6 Pa, the enrobing of a biscuit is perfectly done with the first sample while it fails for the second one. On the latter the chocolate stays as a heap on the biscuit and does not flow down the sides although the fat content of the second sample is 33 % fat and that of the first sample only 27 %. The explanation is found in the different emulsifiers in the samples. The second sample includes only 0.3 % lecithin while the first includes 0.3 % lecithin and additional 0.2 % PGPR (Palsgaard 4125).

Fat bloom on chocolate or chocolate products is a disastrous thing and strong efforts have to be undertaken to prevent this serious form of a quality defect. Reasons for fat bloom on plain chocolate are mainly insufficient precrystallization (tempering) and polymorphic crystal transition due to having created too much unstable crystals during precrystallization or in the cooling tunnel (too cold, too short). In most of the cases of fat bloom fat migration in filled chocolate products is the reason. Weak points are here a high ratio of filling to chocolate, thin stages and bottoms, nut fillings and inlays, incompatible fat combinations, too high and cycling storage temperatures. According to the diffusion equation (Ziegleder, 2012) filling oils are migrating through cocoa butter. As a consequence fat bloom is arising at the chocolate surface. Migration is running linearly with the square root of time. Within the very first time after production there is an intensive oil migration, with decreasing gradient migration slows down. There is a strong influence of storage temperature on oil migration as the diffusion coefficient is higher at higher temperatures. The thinner the chocolate layer (bottoms, stages) the more intense is the oil migration. Only the free mobile oil can migrate. If part of the oil is bound at porous particle surfaces or immobilized due to crystallization it will not migrate. Therefore fat bloom resistance can be increased by using a recipe where the part of free oil is low. Fat migration can be reduced by an adequate geometry of the product. Very important for fat bloom prevention is the storage temperature. It should be low (e.g. 18 °C) and should not cycle between high and low temperatures (Biczo et al, 2005; Biczo-Kabai, 2011). In the case of one-shot or cold formed products there has to be given sufficient time for cooling and stabilization of the chocolate shells before filling.

4. CONCLUSION, SUGGESTIONS

This paper shows the importance of Food Physics on different examples in the food chain from cocoa to chocolate and a confectionery bar. The research of the drying behaviour of Indonesian cocoa and the influence of the drying conditions on the kinetics of the reduction of aroma precursors glucose and fructose showed good results for a drying temperature of 40 °C and an air velocity of 0.2 m/s in a 1-stage drying process and for 60°C/40°C and 0.2 m/s in a 2-stages process. Important is that the drying temperature does not exceed 40°C after reaching a moisture content of 25 % w.b. In the meantime the results have been implemented into model processing units, widespread over Indonesia, consisting of a fermentation unit and a drying unit under one solar roof and supplemented with a biomass oven for the wet season.

From products with a special texture (e.g. wafers, biscuits, cookies) the consumer expects not only a nice eye appeal but also a nice sound when he eats the product. Therefore in the product design a sound design has to be included.

Cutting of hard and brittle products like wafers can be done well with stationery cutting wires located in a frame.

For enrobing of centers (e.g. wafer block) the flow behavior of the chocolate is relevant. By using the proper emulsifier(s) in proper quantities the flow properties can be adjusted well.

Strong efforts have to be undertaken to prevent fat bloom on chocolate and chocolate products, a serious form of a quality defect of the product. In most of the cases fat migration in filled chocolate products is the reason. Possibilities for prevention are given in the recipe, product design and the proper storage conditions. In the case of one-shot or cold formed products there has to be given sufficient time for cooling and stabilization of the chocolate shells before filling. Unfortunately there is no possibility to speed up the line when we want to prevent fat bloom.

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EFFECTS OF DIFFERENT SUBSTRATES ON GROWTH OF *SHEWANELLA XIAMENENSIS*

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SUMMARY

Shewanella xiamenensis is known as iron-reducing bacteria, thus it has potential on application in microbial fuel cell systems. In this study, effects of different carbohydrates (glucose, fructose, galactose, maltose, lactose, saccharose and starch) and organic acids (citric, tannic, lactic and tartaric) on utilization and growth of bacteria were investigated using the optical density change and HPLC methods. The results show that S. xiamenensis grew rapidly on monosaccharide (glucose, fructose, galactose) and disaccharides (maltose and saccharose) carbon sources. In the case of starch and lactose the lag-phase was longer that the cases mentioned above. Bacteria were also able to growth on all investigated organic acids, except tartaric acid, with longer lag-phrase. These results will support technological development of MFC using Shewanella culture.

1. INTRODUCTION

Developing carbon-neutral renewable energy sources is an important research area for alternative power systems (Biffinger, *et al.*, 2012). The production of electricity from different wastewaters would be soon an economically desirable process because of the worldwide population increase and energy supply exhaustion (Hashemi and Samimi, 2012).
In a conventional biofuel process, the chemical energy contained in biomass and organic waste can be recovered in different forms (bio-ethanol, bio-methanol, bio-hydrogen, biogas, *etc.*). To use the conserved energy of the carrier molecules, more step(s) are needed to release the energy (*e.g.* burning) that occur relevant losses. Bioelectricity could be directly generated from wastewater using a microbial fuel cell (MFC). The overall conversion efficiencies of MFCs that can be reached are potentially higher compared to other biofuel processes (Rabaey, *et al.*, 2005).

Shewanella xiamenensis is a marine microbe and firstly isolated from coastal sediment (Xiamen, China). The physiological and biochemical features of this species were similar to those of members of the genus *Shewanella*, and it was most closely related to *S. oneidensis* (Huang, et al., 2010) that now became a frequent-used alternative power microbe in marine applications (Gorby, et al., 2009). Meanwhile many scientific data are available in literature about this organism, so far very few studies are published relating to S. xiamenensis.

In this study, the metabolism of different carbohydrates and organic acids of *Shewanella xiamenensis* are focused.

2. MATERIALS AND METHODS

2.1. Culture and maintenance

Shewanella xiamenensis strain DSMZ 22215 were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) and applied as exo-electron producer. Marine agar and Luria-Bertrani (LB) broth were used for inoculation of bacteria at 30°C.

2.2. Media and growth

Different mono-, (D-glucose, D-fructose, D-galactose,) di- (D-maltose, D-lactose and D-sucrose) and polysaccharides (starch) as well as organic acids (citric, tannic, lactic and tartaric) were supplemented in concentration of 2 $g \cdot L^{-1}$ to growth medium (Marine broth) that consists of 5.84 $g \cdot L^{-1}$NaCl, 0.1 $g \cdot L^{-1}$ KCl, 0.25 $g \cdot L^{-1}$ NH_4Cl, 12 $g \cdot L^{-1}Na_2HPO_4 \cdot 12H_2O$, 2.57 $g \cdot L^{-1}$ $NaH_2PO_4 \cdot 2H_2O$. The growth of the microorganism was followed by monitoring changes of optical density at wavelength of 600 nm (OD600nm) with spectrophotometer.

2.3. Determination of sugars and organic acids

The respective saccharides and organic acids in the sample solution were determined using Thermo Scientific Corporation Surveyor HPLC system. The system consists of quadruped Surveyor pump, Surveyor automatic injector, Surveyor RI and PDA 210 detector and Aminex HPX-87H column (Bio-Rad). Parameters of measurement: time of running was 25 minutes; eluent was 0,005 N sulphuric acid; volume of injection (10 µl) depended of carbohydrate-concentration of samples; the temperature of the column and detector was 45 °C; the flow-rate was 0.5 ml/min.

3. RESULTS AND DISCUSSION

Shewanella xiamenensis strain DSMZ 22215 was able to utilise and grow on wild range of carbon sources (Figure 1). All investigated monosaccharaides (glucose, fructose and galactose) were utilized properly by bacterium. In these cases, lag-phases were short and relevant quantity of cell mass was produced. The microbe also utilized the disaccharides (maltose, lactose and sucrose) very efficiently. Sucrose and maltose were metabolized faster, and in the case of lactose the lag-phase was longer. This result is in agreement with one published by Nagy (2002) and it can be explained that rate of synthesis of □-galactosidase by *S. xiamenensis* cells was low in the initial phase. The growth of bacteria was turned to exponential phase when the level of □-galactosidase was as high as enough to hydrolyze lactose to glucose and maltose. The profile of growth curve (OD600nm) of bacterium in the case of starch as substrate was very similar to lactose case. Starch was quite as complex substrate as to easily to be utilized by bacterium cells. Moreover, in this case the specific growth-rate significantly lower than the case of lactose as substrate

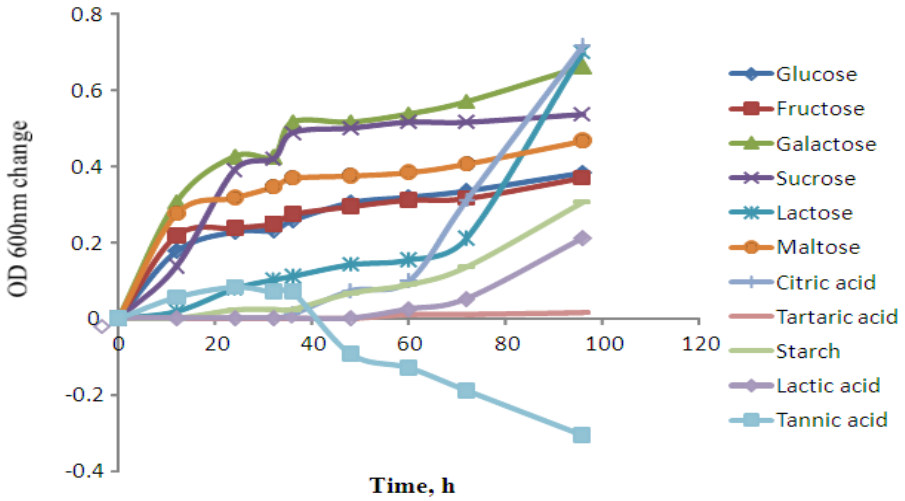

Figure1: OD_{600} change during the experiment

The organic acids were also metabolized by *S. xiamenensis*, except of tartaric acid. Comparing with saccharides generally the long lag-phase and low biomass production was detected. However, citric acid seemed to be good carbon source for production of biomass. Lactic and tannic acid were also metabolized, but they served less effective energy source than the citric acid. In the case of tannic acid the decrease in absorbance at 600nm was monitored meaning *S. xiamenensis* was unable to grow and utilize this substrate.

The consumption rates of individual substrate – calculated by HPLC – are illustrated on Fig. 2. In the cases of sucrose, lactose, galactose, fructose and glucose, the consumption rates were 0.142 g/24 hours, 0.137, 0.130, 0,113 and 0.067 g/24 hours, respectively.

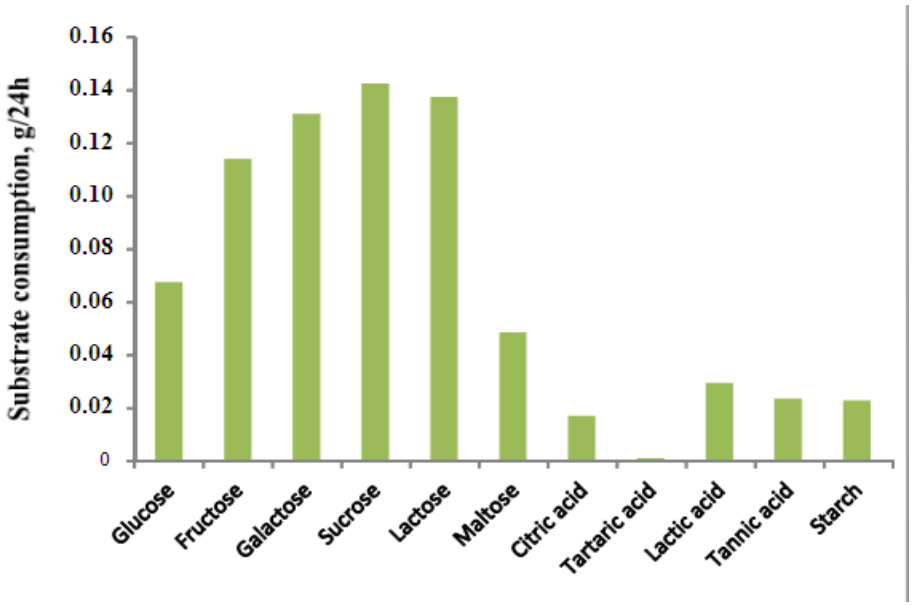

Figure 2: The substrate consumption detected by HPLC technique

The consumption rates of organic acids were definitely lower than the ones of mono- and disaccharides.

4. CONCLUSIONS

Shewanella xiamenensis was able to grow on media supplemented with several sugars and organic acids. Moreover, this species had utilized lactose, galactose, maltose and glucose at high consumption rate, thus it can be applied to develop technology for processing of food industry wastes.

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MARKETING APPROACH OF FUNCTIONAL FOODS

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SUMMARY

The dynamic increase of functional food market raises the interest from increasing number of stakeholders in the food sector. In order to achieve market success, market offers have to satisfy certain conditions. We believe that one of most important criteria of market success is to recognize a relevant and believable customer value. It is important that this customer value should be difficult to copy. This customer value should be clearly distinguished from the values offered by competitors. We have carried out our marketing research keeping these considerations in mind. Approximately 500 respondents have been asked in Budapest, Hungary. The goal of our survey was to contribute to product development and marketing communication.

Values of possible health protecting features of foods have been measured with a special view to consumer knowledge about health effects of possible food additives. This is very important, because our results show that former consumer knowledge about each food additives greatly influences their decision.

Selecting the proper customer values has a crucial importance. We believe that our research can provide important guidelines to product development.

SECTION 1: Agricultural- and food-engineering; Biotechnology and fermentation processes

EFFECTS OF DIFFERENT PRE-TREATMENTS ON DRYING CHARACTERISTICS AND QUALITY OF FREEZE DRIED SOUR CHERRY

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SUMMARY

In this study, the influences of various pre-treatments on the drying kinetics and quality parameters of freeze-dried sour cherry were investigated. Prior to freeze-drying, sour cherry was pre-treated by blanching in boiling water (100°C, 3 and 6 min), immersion in sugar solution (20% w/w, 10 and 20 min), soaking in citric acid (1% w/w, 5 and 10 min) and blanching (100°C) in sugar solution (20% w/w, 3 and 6 min). Freeze-drying of raw samples was taken as a control. The quality of the dried sour cherry was the evaluated in terms of water activity, colour, texture and rehydration. Our research results have shown that all of the pre-treatments can effectively reduce the freeze drying time, the best treatment method is blanching boiling water (6 min). The highest values of hardness and rehydration were found blanching in sugar solution with water at 100°C for 6 min. The better colour retention was observed for samples pre-treated with citric acid solution (1%, 10 min).

1. INTRODUCTION

Research and developments of new preservation methods are continuously carried out in order to improve the quality of the final product. For drying of fruits, freeze drying (lyophilisation) is known to be a good method, by which product shrinkage is eliminated or minimized, and near-perfect preservation results are expected. Freeze drying also prevents heat damage and produces products with excellent structural retention (Aktas et al., 2007).

Energy consumption and quality of dried products are critical parameter in the selection of a drying process. To reduce operating costs of freeze drying, different pre-treatments have evolved. The chemical and thermal pre-treatments have proven to reduce drying time while improving product quality and minimizing energy requirements.

Sour cherries are relatively diverse and broadly distributed around the world. The fruits are generally used for processing purposes, such as for juice, wine and jam. The fruits of sour cherries can also be frozen and dried (Doymaz, 2007). A waxy cuticle covers this fruit, which hinders water transfer and makes dehydration rate very low (Ochoa et al., 2007).

The effects of pre-treatment on some fruits are reported by some authors in the literature. However, there is no or little information regarding the effect of the blanching, citric acid, sugar solution and combined solution on the freeze drying of sour cherry in the literature. The objectives of this paper were to investigate the effects of above mentioned pre-treatments on the drying kinetics, water activity, colour, rehydration ratio and hardness.

2. MATERIALS AND METHODS

2.1. Material

Sour cherry fruits (Újfehértói fürtös) were purchased from a certified organic producer. Fruits were washed, manually pitted, frozen and stored at -20°C prior to drying. The initial moisture content of sour cherry was determined using a forced air oven set (model LP-306, Labor-MIM, Budapest, Hungary) at 105°C, and kept until reaching constant weight. The initial moisture content of the samples was found as 4.52 kg water/kg dry matter (81,9%, wet basis). The final moisture contents of the products for untreated samples and treated ones with blanching, combined solution (soaking time:3 and 6 min), sugar- and citric acid solutions (soaking t.: 10, 20 and 5, 10 min) were 6.1%, 4.8%, 4.6%, 5.3%, 5.35%, 5.93%, 5.95%, 5.2%, 5.1% (wb), respectively.

2.2. Pre-treatments

To reduce the freeze drying time and enhance the quality of sour cherry, four pre-treatments were used before freeze drying. The first pre-treatment was blanching for 3 and 6 minutes in boiling water. Secondly, the samples were blanching in sugar solution (20%, w/w) at 100°C for 3 and 6 min (so called combined). After blanching and combined (blanched in sugar solution) treatment the samples were immediately cooled to room temperature with running water (5 min). The sour cherry samples were immersed in jars of sugar and citric acid solutions, respectively. The sugar concentration was 20% (w/w) and the citric acid concentration was 1% (w/w), respectively. The ratio of material to solution was 1:4 w/w. After range of immersion times (10-20 min and 5-10 min), the samples were blotted with tissue paper, in order to be used for further analysis. Untreated sour cherries were used as a control. Each sample utilized in the experiment weighed 200 g.

2.3. Freeze drying experiments

Drying experiments were carried out using a laboratory scale freeze dryer (model Armfield FT33, Armfield LTD, Ringwood, England), installed in the College of Nyiregyhaza. Weight loss was recorded at 1 min intervals and drying was continued until no mass change was detected. Drying runs were performed at pressures of 40-50 Pa and temperature of 17-20°C. The condenser temperature was set at -54°C during all tests.

2.4. Determination of water activity

Approximately 3 g of chopped dried sour cherries were placed in the sample holder of a Novasina Labmaster (model CH-8853, Novasina AG, Switzerland) a_w-meter, at 25°C.

2.5. Measuring of color

Colours of fresh, pre-treated and dried sour cherry were measured using a ColorLite sph900 spectrophotometer (ColorLite GmbH, Germany). For each sample at least five measurements were made at different positions of the sample and the measured values were compared with those of the fresh sour cherry. Before each test the colorimeter was calibrated on a special white plate. Three Hunter parameters, namely *L* (lightness), *a* (redness/greenness) and *b* (yellowness/blueness) were obtained from each colour measurement. The total colour difference (ΔE) was used to quantify the colour and its changes during treatments (Eq. 1).

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (1)$$

2.6. Texture analysis

The texture characteristics of the pre-treated sour cherries were measured using a CT3-4500 (Brookfield Engineering Laboratories, Middleboro, USA) texture analyzer fitted with a spherical probe (d=4 mm). Compression test was carried out to generate a plot of force (N) vs. time (s). This plot was used to determine the value of hardness.

2.7. Method for rehydration ratio (RR) determination

The rehydration tests were performed at 25°C in distilled water for 120 min. About 5 g dried products were placed in glass beakers containing water. The samples were taken out (when the time reached 120 min) and blotted with tissue paper to eliminate excess water on

the surface. The weights of dried and rehydrated specimens were measured with an electronic digital balance (model JKH-500, Taiwan). RR was calculated as follows (2):

$$RR = \frac{Mass\ of\ rehydrated\ sample}{Mass\ of\ dried\ sample} \quad (2)$$

2.8. Data analysis

All data were analyzed using the analysis of variance (ANOVA). The Duncan's test was used to establish the multiple comparisons of mean values. A statistical program PASW Statistics 18 was used to perform all statistical calculations. All tests were performed in triplicate and the average values were reported.

3. RESULTS

The Table 1 shows the significant effect of pre-treatments on dehydration time, except of sugar solution. In order to improve the drying rate of high moisture content sour cherries with waxy skin layers, pre-treatments prior to drying are considered.

Table 1: Effect of pre-treatments on the drying time of sour cherry under lyophilisation

Treatment	Control (FD)	Blanch. (3 min)	Blanch. (6min)	Combined (3 min)	Combined (6 min)	Sugar (10 min)	Sugar (20 min)	Citric acid (5 min)	Citric acid (10 min)
Drying t. [h]	24^{f}	18^{b}	17^{a}	19^{c}	18^{b}	23^{e}	23^{ef}	20^{c}	21^{d}

^{abcdef} statistical analysis ($p<0,05$) ANOVA Duncan test applies between pre-treatments.

It also reduces the resistance to moisture transport and thereby increasing the drying rate. The sour cherry samples immersed in boiling water (6 min) prior to drying were found to have a shorter drying time than the other pre-treatments and control samples. These results demonstrated that drying times of blanched samples (soaking time: 6min) was about 29,1% shorter than that of untreated samples.

As observed in Table 2, that the blanching and citric acid solution had a significant effect on the water activity of freeze dried sour cherry. The lowest a_w values were observed for citric acid treatment, being between 0.382-0.391.

Table 2: Effect of pre-treatments on the water activity (a_w) for sour cherry

Treatment	Control (FD)	Blanching	Combined (Blanching+sugar)	Sugar, 20%	Citric acid, 1%
Drying t. [h]	$0,469^{c}$	$0,401\text{-}0,413^{b}$	$0,472\text{-}0,481^{cd}$	$0,495\text{-}0,506^{d}$	$0,382\text{-}0,391^{a}$

^{abcd} statistical analysis ($p<0,05$) ANOVA Duncan test applies between water activity.

Microorganisms generally grow best between a_w values 0.99-0.98, while most microbes cease growth at $a_w<0.90$. The a_w of fresh sour cherries is in the range 0.972-0.963. Besides, our results shows that no microbial proliferation and non-enzymatic browning in the treated samples. According to Klewicki et al. (2009) no microbial proliferation occurred when the a_w was $\leq 0,5$ and non-enzymatic browning is most intense at a_w values in the range of 0,6-0,7.

The total colour difference (ΔE) of freeze dried (control) and pre-treated sour cherry are presented in Table 3. The *L*, *a* and *b* parameters of raw material: 59.4, 17.3 and 3.56, respectively. The drying process and pre-treatments changed the values of all parameters. Consequently, citric acid solution ($\Delta E=3,21\text{-}4,62$) was more effective solution for sour cherry.

Table 3: Effect of different pre-treatments on colour, hardness and rehydration ratio (RR) of sour cherry

Quality parameters	Control (FD)	Blanch. (3 min)	Blanch. (6min)	Combined (3 min)	Combined (6 min)	Sugar (10 min)	Sugar (20 min)	Citric acid (5 min)	Citric acid (10 min)
Colour (ΔE, -)	16,82^{h}	8,31^{d}	7,66^{c}	9,86^{e}	10,02^{e}	12,22^{f}	13,41^{g}	4,62^{b}	3,21^{a}
Hardness (Force, N)	9,45^{f}	5,72^{b}	5,56^{b}	5,12^{a}	4,89^{a}	6,36^{c}	6,45^{c}	8,92^{e}	8,54^{d}
Rehydration (RR, -)	2,31^{h}	5,46^{d}	6,04^{c}	6,29^{b}	6,42^{a}	3,19^{f}	4,07^{e}	2,42^{g}	2,55^{g}

^{abcdefgh} statistical analysis ($p<0,05$) ANOVA Duncan test applies between pre-treatments

The all pre-treatments effects were more significant on texture of sour cherries (Table 3). Longer time of immersing gave decreasing in surface hardness of samples (except of sugar solution). As shown in Table 3, the combined pre-treatment (6 min) has the lowest hardness.

The RR of sour cherry at different pre-treatments, calculated from Eq. (2), is shown in Table 3. Rehydration ratios of the pre-treated samples were higher than those of control samples. It can be seen that the rehydration ratio of pre-treated samples with combined solution (soaking time: 6 min) resulted in the highest rehydration.

4. CONCLUSION, SUGGESTIONS

The effect of thermal and chemical pre-treatments on the freeze drying time and quality of sour cherry was studied. The increase in immersing time of blanching and combined treatment decreased the freeze drying time. Blanched (dipping time: 6 min) sweet cherries have shorter drying time than other pre-treatments and control samples. The shorter drying time leads to lower energy consumption and lower drying cost.

The water activity values (a_w) corresponding to these values were under 0.51 for treated sour cherry. These a_w values are recommended for storage of the products under optimal conditions.

The total colour change and hardness of the pre-treated samples were lower than those of the control samples. The rehydration ratio values of pre-treated samples were higher than those of untreated sour cherries. The best pre-treatment is combined solution (soaking time: 6 min) in case of quality of sour cherry. It was established that blanching can reduce the initial number of microorganism, drying time and improve the final texture, water uptake. In contrast with above-mentioned advantages, the blanching process lead to unacceptable microstructure, chemical components and flavours (thermal degradation of products).

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MODELLING THE RHEOLOGICAL BEHAVIOR OF CANDY GUMS

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SUMMARY

Creep-Recovery Test curves were recorded with a Stable Micro System TA.XT-2 precision penetrometer with the 75 mm diameter cylinder probe on candy gums purchased from the local market. The deformation speed of was 0.2 mm/s, the creeping- and recovering time was 60s while the loading force was set to 1N, 2N, 5N, 7N and 10N. Kelvin-, Maxwell-, Burger- and modified Burger models were fitted on the recorded data, then the parameters of the models were evaluated. The calculated parameters can be use for analyzing the occurred changes in candy gum by different effects (like temperature change) and can help to understand and describe better the rheological behavior of candy gums.

1. INTRODUCTION

The candy gums are one of popular confectionery products. Describing quality properties, know and able to manage the changes in candy material, caused by produce technological parameters or component changing, has primary importance from aspect of producer. The quality and quantity of applied gelatin determines the main quality and sensory properties of candy gums (Mohos, 1993).

Lots of research describes properties of different gelatins. Most of them use rotation and/or oscillation viscometry, which give very precise and accurate describe of gelation properties. Montero et al (2002) analyzed gelatins with different origin and found that gel structure stability depends on producing technology. Nevertheless these methods are not appropriate for modeling consumption (biting, chewing). The Creep Recovery Test (CRT) can give a better describe of elastic properties. Dolz et al (2006) characterized the rheological behavior of food emulsions like gels with creep test and give a good model description for these materials. Vozáry et al (2012) investigated the rheological behavior of candy material by different stress strain and deformation speed values, simulating biting. They found, that at higher stress strain and by higher deformation speed the range of elastic parameter of candy gum changes significantly because of cracking of gel structure (Foegeding, 2007).

The aim of our work presented here was to give an appropriate rheological model of candy gums.

2. MATERIALS AND METHODS

2.1. Materials:

The candies contain glucose syrup, sugar, gelatin, dextrose, natural color-, smell and taste components from fruits, bee wax. The material of candy gum has a complex gel structure, the gelation process during the production is occurred by decrease of temperature and pH of hot raw mass after melting into pressed starch powder form. The quality and quantity of applied gelatin has crucial importance. (Mohos, 1993)

For the experiments candy gums purchased from local market were used. The candy gums stemmed from the same production. The candy gums had the same shape with smooth surface. The overall size of the samples was 2 cm x 1 cm x 1 cm with a weight of about 2,40 g. The candies were packed in semi permeable plastic packaging with a weight of 200 g unit. One package contained about 80-84 candies.

The samples were stored in original packaging in a fridge at temperature of 5°C till the time of the measurements. The packages were taken out from fridge and opened just before the measurements.

2.2. Methods:

The experiments were featured in July 2013 with a TA.XT-2 precision texture analyzer (Stable Micro System, UK). 'Force measurement in compression' mode with 'relaxation test' method was used with a cylinder metal plate of 75 mm diameter. The data acquisition rate was 25 points pro seconds. On the samples Creep-Recovery Tests (CRT) were recorded with deformation speed 0.2 mm/s, creeping-recovering time of 60 s, and the value of loading force was 1 N, 2 N, 5 N, 7 N, and 10 N. In every setting 3 sample were measured and evaluated.

The CRT (Figure 1. and 2.) is a complex rheological measuring method, which contains 3 steps. At first the sample is compressed with a definite deformation speed until the determined loading force (F) is reached (D_1). After that the definite loading force is held for a definite time (in our experiments 60 s), it's creeping. At the end of the creeping period the total deformation is the maximal (D_{max}). At the beginning of the third step, called recovering, the loading force is fallen to zero and the relaxation of deformation is measured (E) for the same time as the creeping was endured. At the end of the recovering period the measured deformation is the irreversible plastic deformation (P).

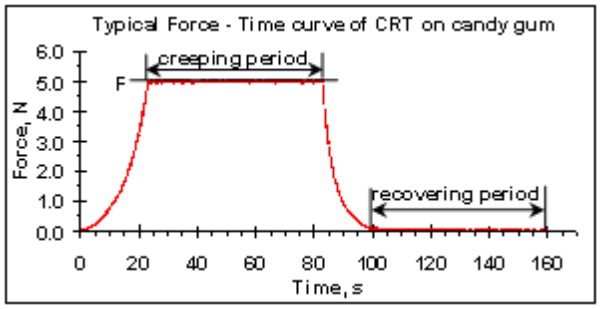

Figure 1: Force – time curve of CRT

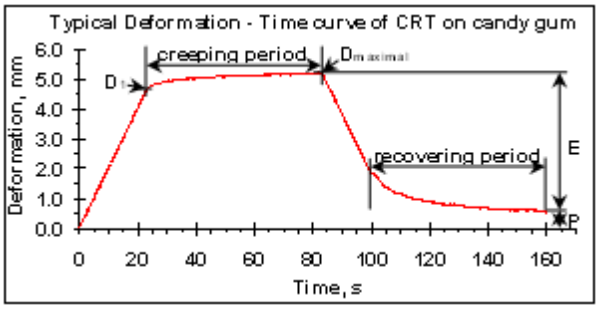

Figure 2: Deformation – time curve of CRT

The recorded force – deformation – time data series of CRT curves were handled with Texture Exponent 1.21., R Studio 0.97.551., and MS Office® Excel 2003 SP3 software. On the creeping section of the curves after a smoothing with Savitzky-Golay method a Burger model was fitted with four coefficients.

The Burger model (Sitkei 1981)(Figure 3.) is a combination of a Maxwell element and a Kelvin-Voigt element, so it contain an elastic element (E_0), which give an instantaneous response for stress strain; a Kelvin-Voigt element (E_r, η), which show a retarded deformation for stress strain; and a viscous element (η_v), which shows the irreversible deformation in the material. The Burger model is developable with further Kelvin-Voigt elements, with which the model is transformed to generalized Kelvin model.

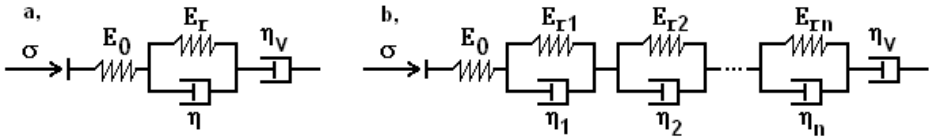

Figure 3. Schematic figure of Burger model (a) and the generalized Kelvin model (b)

The relative deformation is depended on stress according to Equation 1 (Sitkei 1981)

$$\varepsilon(t) = \frac{\sigma_0}{E_0} + \frac{\sigma_0}{E_r}\left(1 - e^{-t/T_r}\right) + \frac{\sigma_0}{\eta_v}t, \quad where \quad T_r = \frac{\eta}{E_r} \qquad \text{(Eq. 1)}$$

The value of stress strain (σ_0) was calculated from the applied loading force and the contact surface area of candy gum sample. So the general form of fitted model was the follow (see Equation 2).

$$\varepsilon(t) = a + b \cdot t + c \cdot \left(1 - e^{-t/d}\right) \qquad \text{(Eq. 2)}$$

The coefficients of Equation 2 were correlated to the loading force values. From the coefficients the parameter of fitted Burger models were calculated and correlated to loading force values. Comparing Eq. 1 and Eq .2 the coefficients of fitted Burger model mean:

$$a = \frac{\sigma_0}{E_0}, \quad b = \frac{\sigma_0}{\eta_v}, \quad c = \frac{\sigma_0}{E_r}, \quad d = \frac{\eta}{E_r} = T_r .$$

3. RESULTS AND DISCUSSION

Description of creeping and recovering of candy material was failed with the Kelvin model, while the modified Burger model with two Kelvin-Voigt element did not give closer fitting. So the Burger model - among the applied rheological models - had the best fitting and the closest correlation with the measured curves. The coefficients of Burger model increased in function of the loading force (Figure 4-7.)

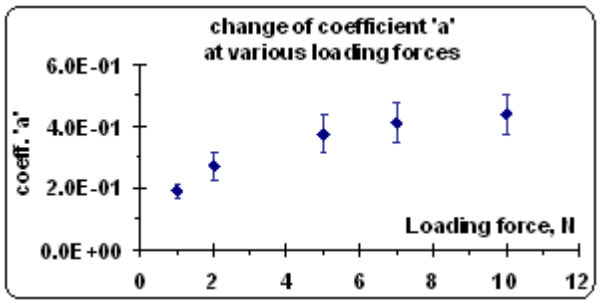

Figure 4: Change of coefficient 'a' at various loading force on candy gum

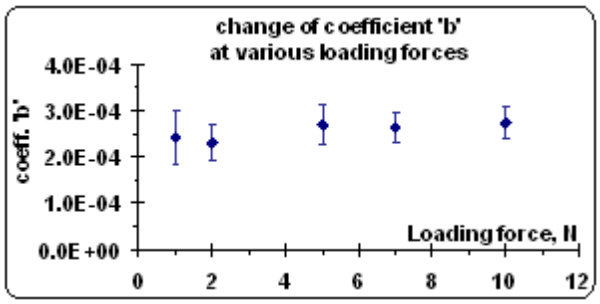

Figure 5: Change of coefficient 'b' at various loading force on candy gum

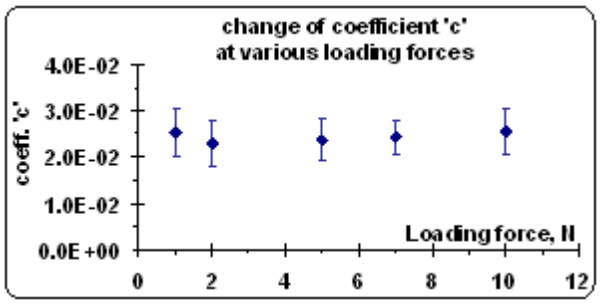

Figure 6: Change of coefficient 'c' at various loading force on candy gum

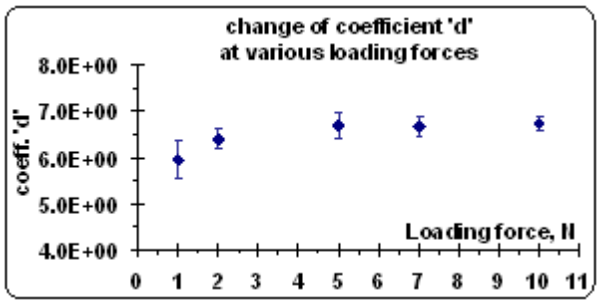

Figure 7: Change of coefficient 'd' at various loading force on candy gum

In case of each parameter of Burger model close linear connection was found with the loading force (Figure 8-11.). The calculated retardation time (coefficient 'd', Figure 7.) was about 6-7 seconds. Regarding the measuring parameters it was found, that the applied loading force has effect on rheological parameters.

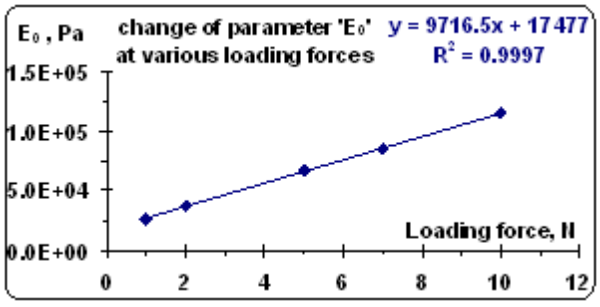

Figure 8: Change of parameter E0 at different loading force values on candy gum

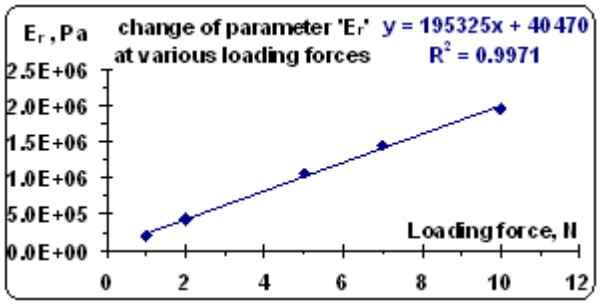

Figure 9: Change of parameter E_r at different loading force values on candy gum

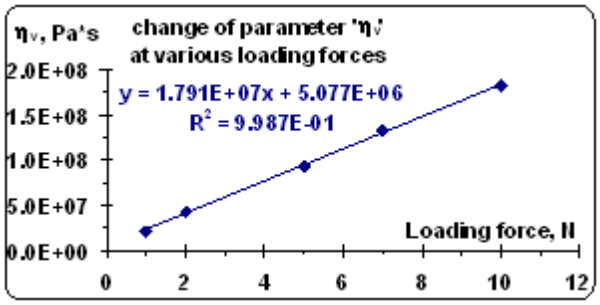

Figure 10: Change of parameter □v at different loading force values on candy gum

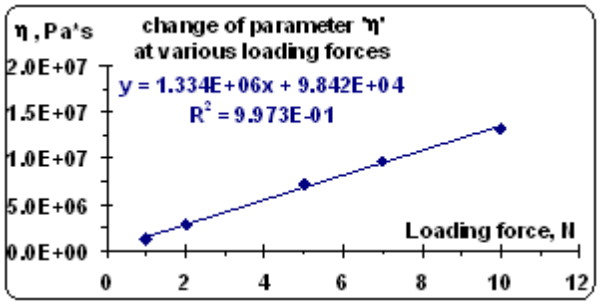

Figure 11: Change of parameter □ at different loading force values on candy gum

4. CONCLUSIONS AND PROPOSAL

The Burger model is available describing the rheological behavior of candy gum. Choosing a good creeping time, loading force and deformation speed the follow up changes (caused by e.g. storage temperature) in candy can be possible. Among the set parameters of measurement the loading force has significant effect on Burger model parameters, here close correlations were found.

Further experiments are advised on one hand to complete the model description, on the other hand to give detailed description of effects of storage conditions, especially temperature.

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EXAMINATION OF ORGANIC EINKORN WHEAT (TRITICUM MONOCOCCUM) AND ORGANIC EINKORN WHEAT BEER HEALTHCARE POTENTIAL

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SUMMARY
The using of einkorn wheat (Triticum monococcum L.) in brewing technology has been occurred at the Corvinus University of Budapest, Department of Brewing and Distilling in 2009. The possibility of growing the einkorn wheat organically give us a possibility for making an organic healthcare beer. After measurements we could confirm, that einkorn wheat antioxidant capacity is higher than other examined grain samples, and the final product antioxidant capacity in most case is higher than the others. The einkorn wheat, its malt and the einkorn beer samples mineral content was higher than other examined samples one.

1. INTRODUCTION

Einkorn (*Triticum monococcum ssp. monococcum* L.) is an ancient wheat and is potential cropped in environmentally friendly organic farming. Compared to common wheat, einkorn is generally more resistant to diseases, and has the ability to withstand drought.

Antioxidants (AO) are ‘‘any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate” (Halliwell, 2007). Antioxidants act in various ways, which include complexation of redox-catalytic metal ions, scavenging of free radicals, and decomposition of peroxides. The intensity of these effects depends on the chemical structure and concentration of the AO present. Natural antioxidants may also protect DNA, protein, and membrane lipids from oxidative damage in biological systems, and provide additional health benefits for disease prevention and health promotion (Halliwell, 2007).

Today the einkorn is used for making of pasta, flour, bread and for animal feeding. The researches regarding using einkorn in malting and beer making has been started at Corvinus University of Budapest, at the Brewing and Distilling Department in 2008. Our aim was to make an organic beer, which contains malted or/and unmalted organic einkorn in proportion of at least 51% of dry matter and to prove its healthcare potential. First we studied the mechanical and chemical properties for malting and brewing aspect, after that we optimized the malting parameters, and we made a preliminary analyze of antioxidant content of einkorn wheat against normal wheat.

2. MATERIALS AND METHODS

We measured winter cultivation (labeled WCW) and optional winter cultivation (OWCW) wheat and its malt (labeled WCM and OWCM) for reference and unhulled einkorn (labeled W.E.) and hulled einkorn wheat (labeled H.E.) obtained from Kőrös Maros Biofarm Ltd., Hungary. All chemicals were purchased from Sigma-Aldrich (Hungary). All reagents used were of analytical grade.

2.1. Determination of mineral contents

The whole and hulled einkorn seeds mineral contents determination was defined with inductively coupled plasma atomic emission spectrometry (ICP-AES) (Thompson, 1983).

2.2. Antioxidant assay

Sample preparation

Seed samples were milled to a particle size of less than 0.5 mm in a centrifuge milling. Beer samples were analyzed after degassing and dilution (if needed). The extraction of antioxidants was made according to (Pérez-Jiménez, et al., 2008) with slight modifications. The extraction was carried out in two principal steps, both step was made twice for better extraction.

Spectrophotometric antioxidant determination assays:

The Ferric Reducing Ability of Plasma (FRAP): The measurements was made according to Benzie and Strain (1996). At low pH, reduction of ferric tripyridyl triazine (Fe III TPTZ) complex to ferrous form (which has an intensive blue color) can be monitored by measuring the change in absorption at 593nm which is therefore, directly related to the combined or "total" reducing power of the electron donating antioxidants present in the reaction mixture. The results were given like µM ascorbic acid/g dry matter.

Total Phenolic Content (TPC) assay: The total phenolic content of extracts was determined using to the Folin-Ciocalteu method (Singelton et al. 1965). We used galic acid (GA) as standard, total phenolic content was expressed as µM GA equivalent/g dry matter.

Determination of free radical scavenging activity by DPPH method: Free radical scavenging activity of the sample extracts was determined spectrophotometrically using the method of Blois (1958). This method is based on the measurement of the reducing ability of antioxidants toward the DPPH radical. The results were expressed in µM Trolox equivalent (TE)/g dry matter.

Trolox equivalent antioxidant capacity (TEAC): The method of Miller et al. (1993) is based on oxidation of ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline)-6-sulphonic acid), what can be measured on 734 nm. The results were expressed in µm TE/g dry matter.

3. RESULTS AND DISCUSSIONS

3.1. Results of mineral content measurement

In table 1 are presented the einkorn's mineral content. As can be seen in the table, the content of calcium is very high, which element is very important in brewing, because the calcium is an important cofactor for brewing enzymes and has an important role in fermentation process. It is similar in case of potassium and magnesium too. By consuming einkorn beer the result reflect that einkorn beer can cover a significant percent of daily mineral needs.

Table 1: Results of mineral content measurement

Mineral	Hulled einkorn calculated on dry weight	Barley calculated on dry weight * *Hopulele (1972)	Unfiltered, unpasteurized einkorn beer	RDA%
	µg/mg	µg/mg	mg/L	%
Ca	997	71,7	24	3
Cu	1,83	0,59	<0,15	-
Fe	49,9	4,86	0,16	2,21
K	1073	346	630	31,5
Mg	573	126	108	28,8
Mn	28,6	1,38	0,22	11
Na	49,4	3,32	70	-
P	330	NA	700	47,2
Zn	17,4	3,57	0,16	1,8

3.2. Results on antioxidant capacity measurements

Seed sample measurements

Examining the einkorn antioxidant capacity against different wheat samples we can conclude that einkorn has a higher antioxidant capacity than other examined wheat samples, as it is reflected in figure 1.

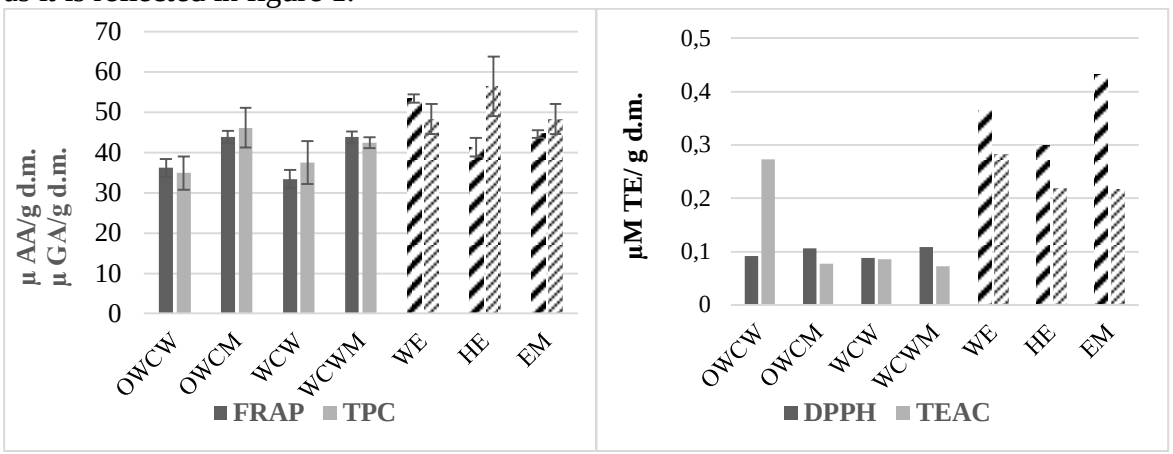

Figure 1 (a,b): Antioxidant measurement results of seed samples

By using different antioxidant assays we got a complex properties of the samples antioxidant capacity. Some antioxidant compound are forming during the malting process, as is shown in figure 1a during the examination of FFRAP and TPC assays. In contrast, the inhibitory compound decrease during the malting process (DPPH and TEAC assays, Fig. 1b).

Examining the final product antioxidant capacity we could conclude, that einkorn beer total phenolic content was the highest against other beer samples. Examining the inhibitory capacity with DPPH antioxidant assay, the einkorn beer has the second highest results.

Beer samples measurement

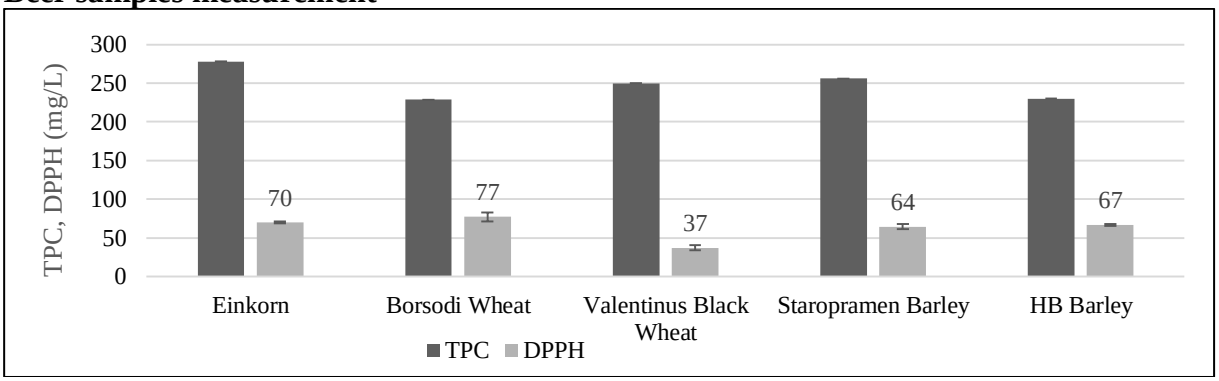

Figure 2: Antioxidant measurement results of einkorn beer and commercial beer samples

4. CONCLUSION

The einkorn wheat tend to have high possibility for use in organic healthcare beer making. The results shown, that einkorn wheat and its malt antioxidant capacity was the highest in all four compared methods against other samples. Having these results, we optimized a brewing receipt, with the usage of 51 % of einkorn wheat and 49 % of barley malt. The final product examination results proved, that the got new product has a higher antioxidant capacity than the other examined Hungarian beer samples from the market. With the use of einkorn in beer making besides generating a new product for the beer lovers, we can increase the healthcare properties of beer.

ACKNOWLEDGEMENT: *We acknowledge for the financial support of the New Széchenyi Plan named ALKOBEER OH-00364/2008, founded by the National Development Agency, and for the TÁMOP-4.2.1/B-09/1/KMR-2010-0005, TÁMOP-4.2.2/B-10/1-2010-0023 project, Hungary.*

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EFFECT OF MICROWAVE TREATMENT OF THE GRAPE MUST FERMENTATION PROCESS

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SUMMARY

Our work during the fermentation process grape must was investigated. In our study we performed a comparison of different treatments (conductive, microwave, yeast, conductive + yeast, microwave + yeast) samples obtained by the fermentation of grape must. During the experiment, control samples also were compared with the results. The fermentation period was studied in samples of sugar, alcohol and acidity changes. Compared to the control sample, the sample was warmed hotplate, a microwave treatment received from the yeast supplement, in addition to yeast inoculation heating also received a hotplate and microwave treatment and yeast fermentation properties of samples is also given supplements. The treatments of the samples decreased faster than sugar, the fermentation time is, at best, approximately 40% shorter.

1. INTRODUCTION

The main task of winemaking technology is to optimize of fermentation process in order to suitable production of wine (EPERJESI et al. 1998). Complex processes are take during the fermentation process, which could influence the process by positive or negative way. However, the controlled fermentation is well directed process with to application of appropriate parameters (CALADO et al. 2002; SABLAYROLLES 2009). During the fermentation the emphasis is mostly on to optimize the alcohol, sugar and acid content (PICKERING et al. 1998; BIACS et al. 2010).

2. MATHERIALS AND METHODS

A method was developed for studying the effects of various treatments in the course of must fermentation. The raw material (must) was treated different ways: 1.microwave treatment; 2.inoculation with yeast; 3.conventional heating; 4.their combination. The results of the treatments were compared in the aspect of alcohol concentration, sugar and acid content.

During the experiments the alcoholic content was determined by Malligand-device, the sugar content of must with spectrophotometer, and acidity by titration (with NaOH). The measurements were performed with three repetitions.

3. RESULTS AND DISCUSSION

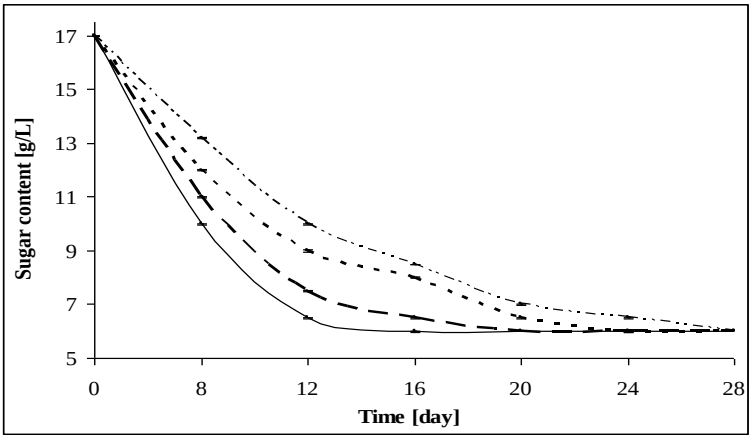

Figure 1. Changes the sugar content of the must during the fermentation of the control (-·-·-), the microwaves (- - -), the yeast (—·—), and the yeast and microwave treated (——) samples.

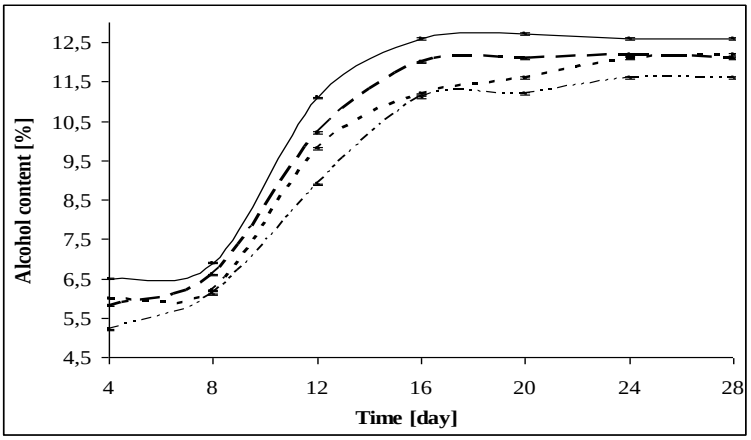

Figure 2. Changes of alcohol content of the must during the fermentation of the control(-·-·-), the microwaves(- - -), the yeast (—·—) and the yeast and microwave treated (——) samples

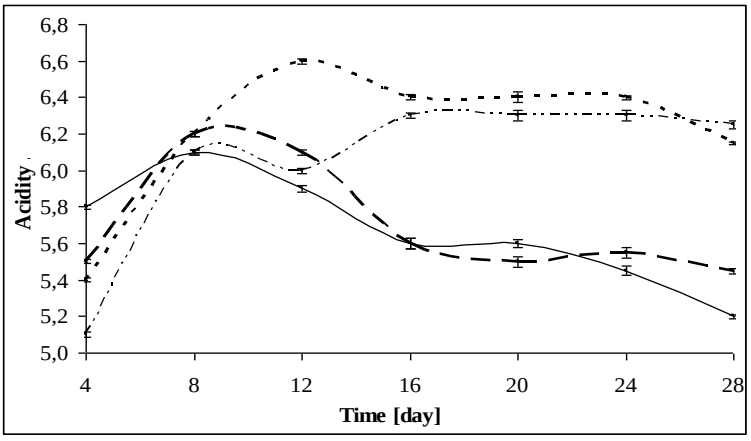

Figure 3. Changes of acidity of the must during the fermentation of the control (-·-·-), the microwaves(- - -), the yeast (—·—) and the yeast and microwave treated (——) samples

The initial sugar content was 17 during the measurements. The difference is visible between no treatment and the treated samples in the 8th day of fermentation (Figure 1). The sugar content degree reduce of the control sample is much slower.

Samples treated with microwave and yeast and inoculated only with yeast samples reached the highest alcoholic content (12.6%, 12.2%) on 20th day of fermentation, which implies that the treatment significantly influence the speed of fermentation.

In simple microwave treated sample achieved the highest alcohol content on 24-28th day of fermentation (12.1-12.2%).

Based on these results, can be stated that the fermentation is significantly influenced by the treatments.

At the beginning of the fermentation acidity was increased for a while and then decreased, as it is written in other studies (KÁLLAY 2010). This can be clearly seen in our measurements (*Figure 3*).

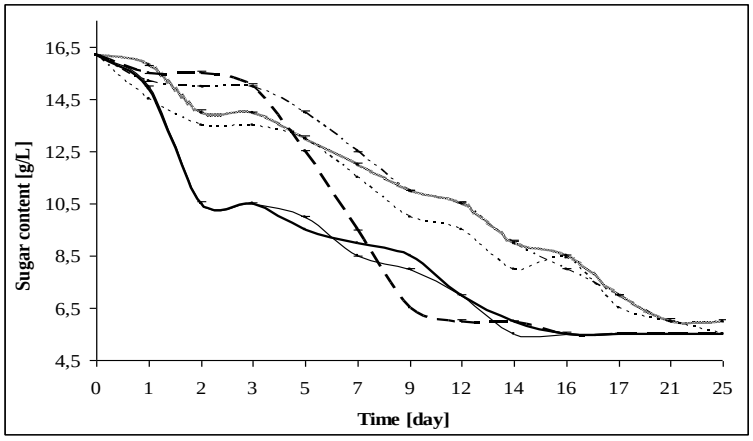

Figure 4. Changes of sugar content of the must during the fermentation of the control (– – – ·), conductive (〰〰), the microwaves (· · · · ·), the yeast (— · —), the yeast and conductive (——), and the microwave and yeast treated (— · —) samples.

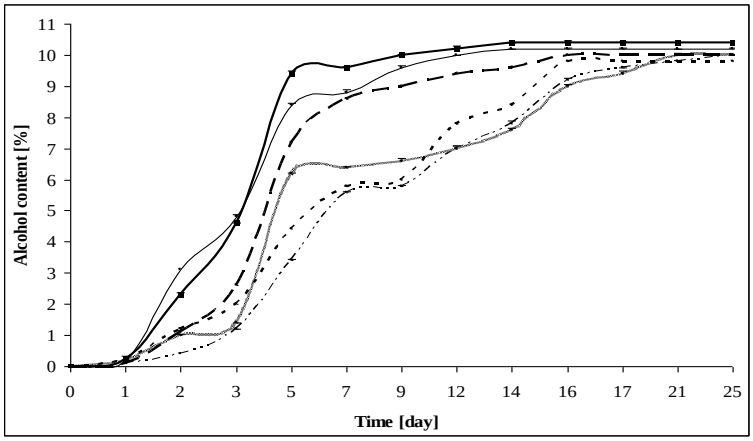

Figure 5. Changes of alcohol content of the must during the fermentation of the control (– – – ·), conductive (〰〰), the microwaves (· · · · ·), the yeast (— · —), the yeast and conductive (——), and the microwave and yeast treated (— · —) samples.

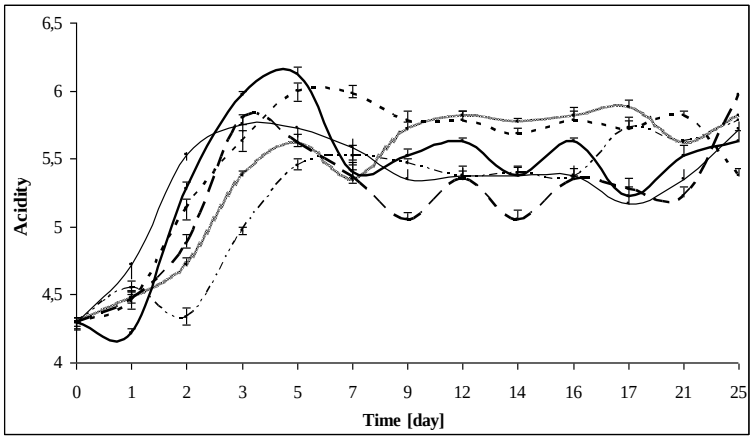

Figure 6. Changes of acidity of the must during the fermentation of the control (– – – ·), conductive (〰〰), the microwaves (· · · · ·), the yeast (— · —), the yeast and

Further experiments, conventional (conductive) is treated by placements. Compared to the control sample, the sample was warmed hotplate, a microwave treatment received from the yeast supplement, in addition to yeast inoculation heating also received a hotplate and microwave treatment and yeast fermentation properties of samples is also given supplements.

Figure 4. shows that the "microwave + yeast" and "yeast + conductive" reached a maximum alcohol content earlier (fermentation 14 days) than the other samples.

The alcohol content of the combined treated samples reached the highest level (10.4% - hot plate + yeast; and 10.2% - microwave + yeast) on 14th day of fermentation. The alcohol content of the must samples that were treated only with yeast inoculation or hot plate reached the highest level on the 18th day of fermentation (10% and 9.8%).

The acidity is similar as in the first measurment. At the beginning of the fermentation acidity was increased for a while and then decreased.

4. CONCLUSIONS

The fermentation time was by 40% shorter in the best case. These results are probably caused by the yeast inoculation and the microwave treatment.

There is no significant difference between the conventional heating and microwave treatments in case of using yeast.

In this case the non-thermal effect of microwave is not prevail or has no effect to measure. It is stated that a short-term heat treatment prior to fermentation at max. 32°C influences the parameters of the fermentation in a positive way by using yeast. The fermentation time is shortened, while the alcohol yield increased.

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CHANGE OF MECHANICAL PROPERTIES AND TASTE ATTRIBUTES OF CARROT (DAUCUS CAROTA SUBSP. SATIVUS) DURING NON-IDEAL STORAGE CONDITIONS

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SUMMARY

During the presented experiments the change of mechanical and taste attributes of carrot samples were tested under non-ideal storage conditions.
Therefore, Nantes type, Nevis cultivar carrots were analyzed by quasistatic (cutting test and creep recovery test) and dynamic firmness (stiffness) measurements methods, electronic tongue and sensory analysis.
Close linear correlation was found between the cutting force - cutting deformation ratio and the mass loss. Furthermore, acceptable exponential correlation was found between the decompression work - compression work ratio and the mass loss in the analyzed interval. Dynamic firmness tests were used for non-destructive measurements. Very close exponential correlation was found between the acoustic stiffness coefficient and the mass loss. The samples were smashed to get juice from the carrots to be measured by the electronic tongue. Sensory attributes were predicted well based on the results obtained with mechanical and electronic tongue tests by the means of PLS regression.

1. INTRODUCTION

Quality is a complex concept, which was determinated by external (color, aroma, size, shape) and internal (taste, texture, ingredients, nutritional value) characteristics, consumer expectations, the market supply and earlier experience (Abbott, 1999).

De Roeck et al (2008) performed cutting tests by Texture Analyzer using a sharp blade on carrot disks treated by high pressure and processed at high temperature. The firmness of the samples treated by high pressure was higher than the samples processed at high temperature.

Zude et al (2006) analyzed stiffness of apple with acoustic method at 4°C temperature and 90% RH in fridge and controlled atmosphere storage. The stiffness coefficient was determined using the first peak of resonance frequency and the mass. The parameter showed good correlation with the texture of the fruit and the sensory measurement.

Kovács et al (2012) analyzed five different carrot juices with electronic tongue and sensory analysis. The panelists found significant differences in the certain characteristics of juices, such as appearance, aroma, taste attributes. Principal component analysis plot calculated from the electronic tongue results showed a clear separation between the sample groups, with a ranking on sourness similar to the one from the panel.

Sensory and quality attributes of raw, vacuum-treated and boiled carrots were tested (Araya et al, 2009). 19 quantitative differences of aroma, appearance, texture, aftertaste was described by 10 trained panelists.

Objective of the work was to find relationship between the mass loss, furthermore mechanical and taste attributes. In addition, our aim was the prediction of sensory attributes based on the mechanical parameters and the electronic tongue results.

2. MATERIALS AND METHODS

2.1. Materials

During the experiments Nantes kind of type, Nevis variety carrots were measured. The experiments were performed in three consecutive years, between 2008 and 2010 after harvest.

Furthermore the experiment of years in 2008 and 2009 were stored under „non-ideal" conditions in refrigerator, where the forced air flow was applied at the middle and top part of the refrigerator. The measurements were performed at the beginning of storage and later once a week. The samples of experiment in 2010 after the harvest were stored under long term („ideal") storage conditions in refrigerator as well, and in same time this the last measured group was placed into another chamber, where was short term, („non-ideal") condition. Each week a group was transferred into the non-ideal storage chamber, however the control group stayed under ideal ambience conditions during the whole storage time. The storage period of the different carrot groups under non-ideal conditions was as follows: 4 weeks for group No. 4, 3 weeks for group No. 3, 2 weeks for group No. 2, 1 week for group No. 1. The carrots were grouped at the beginning of storage. The storage conditions were as follows: experiment (2008, 2009): temperature: 8.0±0.5°C; relative humidity: 84±2%, experiment (2010): „Ideal" storage: temperature: 2.0±0.5°C; relative humidity: 96±2%, „Non-ideal" storage: temperature: 8.0±0.5°C; relative humidity: 84±2%.

2.2. Methods

During the experiments the mass was measured at first. After this the stiffness coefficient was measured by acoustic response method which provides information on internal firmness of the sample. The stiffness coefficient was calculated by the formula, as follows: $s = f^2 \cdot L^2$ [m^2s^{-2}] where f – resonance frequency [Hz], L – length of the sample [m] (Zsom-Muha & Felföldi, 2007).

The (cutting force)/(cutting diameter) ratio and the (decompression work) /(compression work) ratio were calculated from measurements performed by SMS Texture Analyzer. 5 mm thick disks were cut out of the carrots at the 1/3 of length from the top end. The disk was cut along its diameter (0.1 mm/s cutting speed, maximal deformation 8 mm, sharp blade of 3 mm thickness, and three cutting tests were performed on each carrot). (Decompression work)/(compression work) ratio was determined from the following parameters: the ratio of work from zero force up to maximum cutting force to the work from maximum cutting force up to maximum distance.

Creep recovery test was performed by SMS TA-XT2 type precision penetrometer and ratio of loading force to deformation before creep and ratio of elastic deformation to maximal deformation were determined. 9x9x9 mm length cubes were cut from both of xylem and phloem carrot parts. The cubes were compressed with 75 mm diameter aluminum plate. The measuring parameters were as the follows: 60 N loading force, 60 s creeping time, 0.1 mm/s speed of measurement.

Alpha ASTREE II potentiometric Electronic Tongue (Alpha M.O.S., Toulouse, France) was used to measure the taste attributes during the storage. The sensors perform global taste analysis similar to the human tongue's taste receptors (Kovacs, Kantor & Fekete 2008).

The sensory analysis was performed by 16 panelists in experimental year 2010. The panelists got one „carrot disc" from middle part of carrots in case of all of five groups of storage time, and tested it, according to the following attributes: „good odour", „orange colour

intensity", „bite and chewing", „sweet taste", „bitter taste", „global impression". The evaluation method was the ranking test. The results were evaluated by Page test.

3. RESULTS

Close correlation was found between the cutting force/cutting diameter and the mass loss in experimental year 2008 (R^2 = 0.8523). This result was confirmed by results of 2009 and 2010 years. The tendency was similar in each of case. The common fitting of model for the all measuring years showed close correlation as well, that the figure 1 shows.

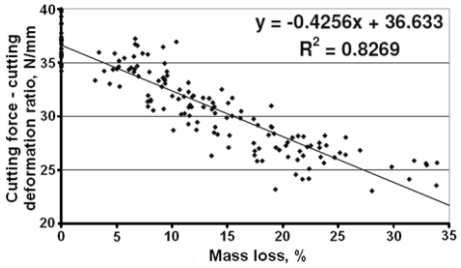

Figure 1: Cutting force/cutting diameter ratio (F_v/D_v) versus mass loss (common fitting to the all of experiment, 2008, 2009 and 2010)

Good correlation was found between the decompression work/compression work ratio and mass loss when the ratio was analyzed with exponential model. The result was confirmed by experiments in 2009 and 2010. Good correlation was found in the case of common fitting to the all of experiment; however the coefficient of determination was worse than the results in each years (figure 2). The results were confirmed by Durbin-Watson statistic, value of Akaike criterion and prediction error as well.

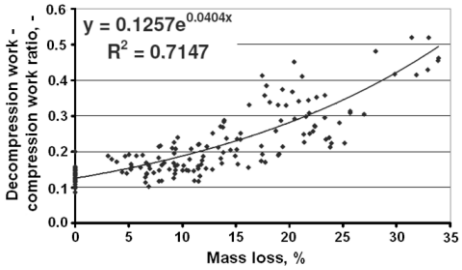

Figure 2: Decompression work and compression work ratio versus mass loss (common fitting to the all of experiment, 2008, 2009 and 2010)

Exponential relationship was found between the acoustic stiffness coefficient and mass loss in 2008 and 2009 and 2010. The model was confirmed by DW statistic, the R^2, the AIC and RMSE values. The each experiment showed close correlation between the parameters, however the common fitting showed weak result because of the high deviation. Figure 3 shows the results of year 2008. During the storage the firmness decreased exponentially.

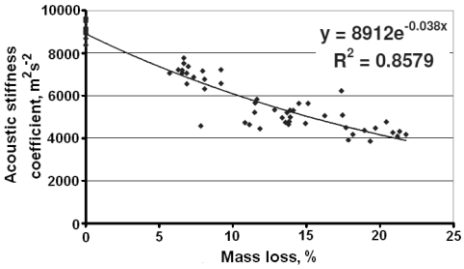

Figure 3: Acoustic stiffness coefficient versus mass loss (experiment in 2008)

The Page-test results showed significant ranking for the „bite and chewing", „sweet taste" and „global impression" sensory attributes, which ones were predicted with PLS-regression by mechanical methods and electronic tongue results (table 1). The „bite and chewing" was predicted from results of the acoustic stiffness coefficient, cutting force - cutting deformation ratio, loading force - deformation before creep ratio and elastic deformation - maximal deformation ratio. There was a close relationship between the predicted and measured variables. The „sweet taste" was predicted from the electronic tongue measurement results. This prediction is confirmed by close correlation of cross-validation and good root mean squared error of prediction. The „global impression" was predicted from the applied parameters at prediction of the "bite and chewing" and "sweet taste" attributes.

Table 1: PLS-calibrations and cross-validation for the sensory attributes of carrot samples based on acoustic stiffness coefficient, cutting force/cutting diameter ratio, loading force/deformation before creep ratio, elastic deformation/maximal deformation ratio and the electronic tongue results

Sensory attributes	LV (No)	calibration				cross-validation				
		r[1]	SEC[2]	RMSEC[3]	Bias[4]	r[1]	R^2	SEP[5]	RMSEP[6]	Bias[4]
Bite and chewing [A]	6	0.96	2.88	2.84	0.08447	0.97	0.95	2.29	2.25	-2.098e-6
Sweet taste [B]	4	0.97	1.82	1.78	-5.37e-5	0.95	0.90	2.36	2.32	-0.067
Global impression [C]	13	0.98	1.69	1.66	4.62e-6	0.93	0.86	3.19	3.14	-0.215

[1]Correlation coefficient; [2]standard error of calibration; [3]root-mean-squared error of calibration; [4]systematic difference between predicted and measured values; [5]standard error of prediction; [6]root-mean-squared error of prediction. [A]prediction based on acoustic stiffness coefficient, cutting force/cutting diameter ratio, loading force/deformation before creep ratio and elastic deformation/maximal deformation ratio [B]prediction based on electronic tongue results. [C]prediction based on acoustic stiffness coefficient, cutting force/cutting diameter ratio, loading force/deformation before creep ratio, elastic deformation/maximal deformation ratio and the electronic tongue results. LV: latent variable

4. CONCLUSIONS

Close linear correlation was found between the cutting force - cutting deformation ratio and the mass loss. Acceptable exponential correlation was found between the decompression work - compression work ratio and the mass loss in the analyzed interval. Close exponential correlation was found between the acoustic stiffness coefficient and the mass loss. Sensory attributes were predicted well based on the results obtained with mechanical and electronic tongue tests by the means of PLS regression. The selected mechanical parameters and electronic tongue were suitable for prediction of the changes in definite sensory attributes.

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EFFECT OF MEMBRANE MODUL VIBRATION ON DAIRY WASTEWATER TREATMENT

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SUMMARY
In this study high shear rates were utilized at the membrane surface in order to reduce membrane fouling. The performance of a vibratory shear-enhanced processing system for ultrafiltration, nanofiltration and reverse osmosis membrane filtration was investigated during purification of real dairy process wastewater came from dairy industry. In the first part of the experiments, short-term tests were carried out, where the effects of vibration amplitude and recirculation flow rate were compared. The permeate flux, turbidity, conductivity and chemical oxygen demand reduction were investigated with vibration and non-vibration methods using the same membranes and operational parameters. Furthermore, in the second part of the experiments, concentration tests were carried out. Scanning electron microscope pictures showed that the vibration method has better performance, since it can be attributed to its higher membrane shear rate which reduces solids concentration at membrane and its transmission.

1. INTRODUCTION

Among the food industries the dairy industry requires and eventually discharges huge volume of water, with wide fluctuations in the quality of effluent (Farizoglu & Uzuner, 2011). Several works have indicated that nanofiltration (NF) and reverse osmosis (RO) are convenient operations for the treatment of effluents at source and achievement of the set targets (Balannec et al., 2002; Luo et al., 2011). In recent times, the development of membranes with high flux and rejection characteristics has enhanced the possibilities of water reuse and recycling. Unfortunately, membrane fouling and the resulting decline in flux, still remains a major bottleneck preventing wide spread application. In order to solve this problem, many researchers have investigated the potential applicability of rotating and vibration modules in wastewater treatment (Shi & Benjamin, 2011). In order to control the decline in flux during the concentration of dairy effluents, a vibration method could be use. The very few reports relating to the treatment of dairy wastewater by vibratory shear-enhanced processing (VSEP), what had shown that NF or RO is adequate for the concentration of milk components (Luo et al., 2011). In the present study, the performances of a VSEP system for the UF, NF and RO of industrial dairy wastewater were investigated, and the vibration and non-vibration methods were compared.

2. MATERIALS AND METHODS

The feed dairy industry wastewater was provided by Sole-Mizo Ltd. (Szeged, Hungary). The main characteristics of the feed wastewater are 1.3 mS cm^{-1} of conductivity, 1150 NTU of turbidity, 6175 mg L^{-1} COD and 6.62 of pH. The wastewater came from a mixture tank from the cleaning of transport lines and equipment between production cycles, the cleaning of tanks, and the washing of milk silos and other equipments. The conductivity and pH were measured with a multi-parameter Consort C535 analyser (Turnhout, Belgium). The turbidity of the samples was determined with a HACH2100N turbidimeter (Loveland, Colorado, USA).

The filtration module was a VSEP Series L (Emeryville, California, USA), equipped with a single circular membrane with a surface area of 503 cm^2 with 13.5 cm outer and 4.7 cm inner radius. As a result, the housing containing the membrane oscillates azimuthally with displacement amplitude, which was adjusted to be 2.54 cm on the outer rim at the resonant frequency of 55 Hz. For UF polyethersulfone 10 000 Da, for NF 240 Da thin film composite and for RO 30 Da thin film composite membranes were used.

Scanning electron microscope pictures were performed with a Hitachi S-4700 field emission SEM (Dallas, Texas, USA) operated at an acceleration voltage of 10 kV in ultrahigh resolution mode. To analyze the gel layer after UF and NF concentration tests with the vibration and non-vibration methods different magnification pictures were recorded and compared. All measurements of VSEP were carried out at 50 ± 1 °C. Before the measurements, the membranes were prepressured with deionized water for 60 min in order to remove the excess of preservation chemicals attached to the new membranes. In short-term tests, with full permeate and retentate recycling were performed. In concentration tests the thermostatic controlled feed tank was filled with 10 L wastewater, the feed pump was started and the vibration frequency was adjusted. When the desired parameters (vibration amplitude, temperature, recirculation flow rate and transmembrane tressure) were reached and had stabilized (under a pressure of 0.8 MPa for UF, 2 MPa for NF and 3 MPa for RO) the permeate was collected separately in order to increase the volume reduction ratio (VRR) till the end of the experiments, when VRR reached 5. Recirculation flow rate (q_V) was constant at 15.14 L min^{-1} during all concentration tests.

3. RESULTS AND DISCUSSION

The high shear rate on the surface of the membrane is caused by both recirculation flow rate and membrane vibration. In short-therm tests, the increasing recirculation flow rate from 7.57 to 15.14 L/min increased the flux. Higher recirculation flow rate causes higher shear-induced back-diffusion and can decrease the concentration of solutes and the precipitation of particles on the membrane, thereby reducing the extent of membrane fouling, with resulting higher fluxes. Although the shear rate can be increased by elevating recirculation flow rate this induces large pressure drops in the modules. The creation of a more effective high shear rate on the membrane without a pressure drop would be possible with a moving part such as the high tangential vibration of the membrane module with increasing vibration amplitude. Higher increasing vibration amplitude also increased permeate flux. Probably higher vibration amplitude can reduce solutes accumulation at the surface and leading to higher permeate flux at a constant TMP due to decrease osmotic pressure differences across the membrane. Furthermore, the vibration increased the rejection values in all cases, but it was more emphasized with the highest porous UF membrane. Turbidity analysis shown that the membrane rejections were always higher than 99.33% (with permeate value of 7.7 NTU), what was the lowest one for UF, resulting transparent, visually clear permeates. In short-term UF experiments, the highest permeate flux was 83 L/m^2–h (at 0.8 MPa) which yielded 380 mg O_2/L permeate COD (R_{COD}=93.85 %). In NF the highest permeate flux was 96 L/m^2–h (at 2 MPa) which yielded 22 mg O_2/L permeate COD (R_{COD}=99.64 %) and in RO the highest permeate flux was 112 L/m^2–h (at 3 MPa) which yielded 3 mg O_2/L permeate COD (R_{COD}=99.95%). From the permeate conductivity, the rejection of salt were calculated. The rejection values increased, due to the lower salt content in the permeate side, when vibration amplitude increased, resulting higher shear rate on the membrane surface. The retained salts lead higher osmotic pressure in the retentate side of the NF and RO membrane resulted higher rejections.

In UF and NF concentration experiments, the permeate was not returned to the feed tank, and 10 L of industrial dairy wastewater was concentrated to 2 L. VRR during the concentration tests of the wastewater treatment was determined as the ratio of the feed volume at the beginning of operation (V_{feed}) to the retentate volume after a certain time (V_{conc}) (Krstic et al., 2007). To know the flux decline, the relative flux (J_{rel}) was calculated via J/J_{water}.

In the concentration tests, the vibration methods resulted in higher shear rates, with almost two times larger values of flux and this trend was independent of membrane type. In NF, flux was pressure-limited and its decay was mainly due to the rise in osmotic pressure. The decay in flux is shown in Figs. 1 and 2. At the end of the concentration tests, relative flux

had decreased to 0.57 and 0.29 for UF, and to 0.38 and 0.09 for NF with the vibration and non-vibration methods, respectively, as VRR increased from 1 to 5.

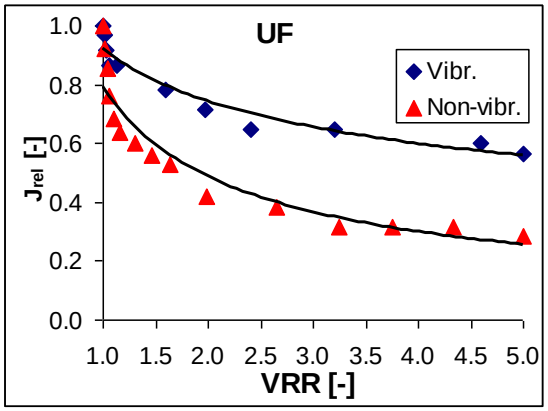

Figure 1: The effect of vibration on the profiles of the relative flux decreasing during ultrafiltration (T=50±1°C; TMP=0.8 MPa, q_V=15.14 L/min, $A_{vibr.}$=2.54 cm)

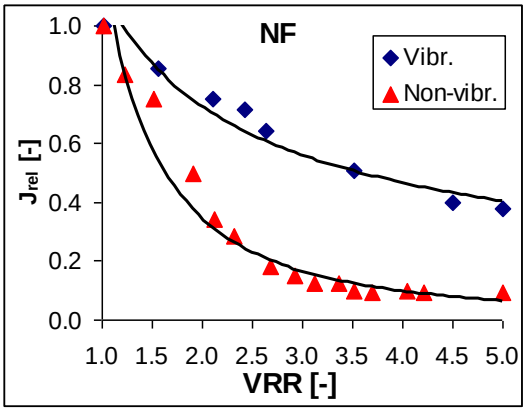

Figure 2: The effect of vibration on the profiles of the relative flux decreasing during nanofiltration (T=50±1°C; TMP=2 MPa, q_V=15.14 L/min, $A_{vibr.}$=2.54 cm)

Variation of conductivity with volume reduction ratio

Good overall salt retention, as measured by the difference in conductivity between the permeate and the retentate, was achieved in both the UF and the NF concentration tests. The conductivity slowly increased continuously and only a small difference was observed between the values of the vibration and non-vibration methods. During the NF test, the permeate conductivities with the non-vibration method were higher than those with the vibration method, due to the lower retention rates. Since the vibration changed the structure of the gel layer on the surface of the membrane, the ion transmission through the pores also changed.

Membrane fouling examination by scanning electron microscope

SEM images of the scale that formed on the membrane surface during the concentration tests of dairy wastewater are shown in Figure 3. With the non-vibrating method, the membrane was almost uniformly covered with scale (NV_UF; NV_NF), but with the vibration method the morphology of the scale layer changed (V_UF; V_NF). The scale in UF appeared mostly continuous relative to NF, and the scale morphology changed from a tightly packed layer to a scattered one with more open spaces between individual clumps. To examine the differences in the NF gel layer structure with the vibration and non-vibration methods, SEM pictures at higher magnification were also taken. These results show that the vibration changed the structure and average particle diameter of the gel layer.

4. CONCLUSIONS

In this work the performance of a vibratory shear-enhanced processing (VSEP) system for ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) membrane filtration of industrial dairy wastewater was investigated. Vibration of UF, NF and RO membranes in an L-mode VSEP system reduced membrane fouling. For short-term tests vibration reduced the rates at which the permeate flux declined, increased the practical recovery. Treatment with vibration led to rejections of most ions >30.7 % for UF, >76.6 % for NF and >98 % for RO. It may be concluded from these studies that, each individual, single UF, NF and RO treatment could improve a good treatability of dairy industry wastewater, but NF and RO could generate treated effluents that met the strict requirement of Hungarian COD threshold limit below 150 mg O_2/L:

COD [mg O_2/L]	UF	NF	RO
non-vibration	1181.9	134.6	16.1
vibration method	380.0	22.0	3.0

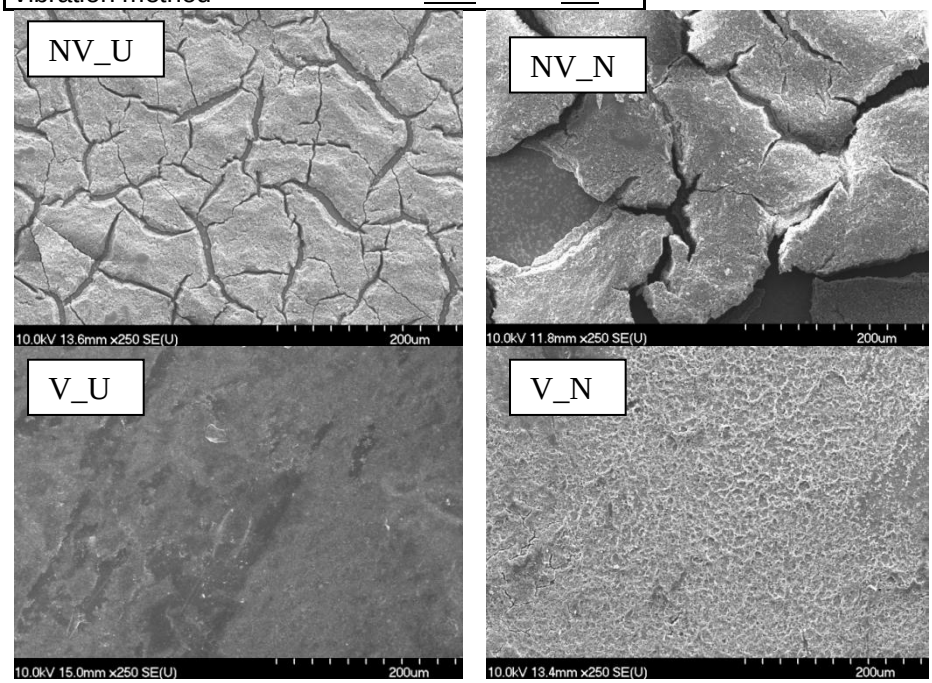

Figure 3: SEM images of UF and NF membrane without vibration (NV_UF; NV_NF) and UF and NF membranes fouled at vibration amplitude of 2.54 cm (V_UF; V_NF) and after the concentration tests. All specimens were taken from a location 10 cm from the center of the membrane.

The results showed that NF and RO single membrane operations allowed the purified water to release in the environment, but UF permeate water was not reached the COD Hungarian standard.

For concentration tests of UF and NF using vibration method greatly reduced the membrane fouling mainly with gel layer reduction. SEM images indicated that on the membrane surface were almost uniformly covered with scale forming gel layer in the non-vibrating methods in both UF and NF system, but in the methods with vibration, the morphology of the scale layers were different. The scale in UF appeared mostly continuous, compare to NF, and it becomes more scattered with more open space between individual clumps. A higher magnification of SEM pictures showed that the scale in NF non-vibration method formed a more aggregated and continuous, overcrowded layer, whereas the scale in the vibration method comprised a less number of smaller and mainly only one layer round-like particles. Compared with UF process NF had a higher efficiency and less membrane fouling.

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EXTRACTION OF THE ACTIVE SUBSTANCES OF THE STINGING NETTLE (URTICA DIOICA L.) BY SOXHLET- AND SUPERCRITICAL EXTRACTION

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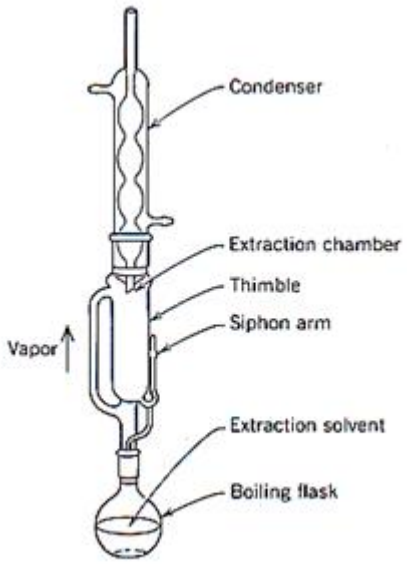
SUMMARY

Stinging nettle *(Urtica diodica L.) is a well known weed and herb. It can be found everywhere in Hungary. It prefers nitrogen rich soil, for example a planted robinia woodland is an ideal habitat for it. Its main active ingredients are: flavonoids, cinnamic acids, minerals, vitamins (beta-carotene, C, B and K vitamins), ascorbic acid, formic acid, acetylcholine, histamine, serotonin and chlorophyll. Soxhlet extraction The process of transferring the partially soluble components of a solid to the liquid phase using a Soxhlet extractor. The solid is placed in a filter paper thimble which is then placed into the main chamber of the Soxhlet extractor. The solvent (heated to reflux) travels into the main chamber and the partially soluble components are slowly transferred to the solvent .Supercritical extraction is suitable for careful extraction of herbs' active ingredients. As a solvent, usually carbon dioxide is used, because it is cheap, not toxic, its critical temperature is very low (so substantial heat damage can be avoided), and at the end of the procedure it can be perfectly separated from the dissolved ingredients.*

1. MATERIALS AND METHODS



Drog extracion in Soxhlet extractor is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. (Fábry, 1995)

Normally a solid material containing some of the desired compounds is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The extraction solvent to be used is taken into a distillation flask and the Soxhlet extractor is now placed onto this flask. The Soxhlet is then equipped with a condenser.

The solvent is heated to reflux. The solvent vapor travels up a distillation arm, and floods into the chamber housing the solid. The chamber containing the solid material is slowly filled with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask.

The solvent is heated to reflux. The solvent vapor travels up a distillation arm, and floods into the chamber housing the solid. The chamber containing the solid material is slowly filled with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask.

This cycle may be allowed to repeat many times, over hours or days. During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead

of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled.

After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded.

Supercritical extraction is suitable for careful extraction of herbs' active ingredients. As a solvent, usually carbon dioxide is used, because it is cheap, not toxic, its critical temperature is very low (so substantial heat damage can be avoided), and at the end of the procedure it can be perfectly separated from the dissolved ingredients.

Supercritical fluids can be used to extract components from samples. The main advantages of using supercritical fluids for extractions is that they are inexpensive, contaminant free, and less costly to dispose safely than organic solvents.

Supercritical fluids are the materials which combine useful properties of gases and liquids. They behave like gas and liquid in terms of different features. They are the phases at the critical temperature and critical pressure of the materials.

When a material is heated until its specific critical temperature in a closed system, a dynamic equilibrium, which includes the same number of molecules coming out of liquid phase to gas phase by gaining energy and going in to liquid phase from gas phase by losing energy, is obtained. At this particular point, the phase curve between liquid and gas phases disappears and supercritical material appears. (Cossuta, 2008)

Diffusivity for SF is 100 times more than liquids and 1,000 - 10,000 times less than gases. Viscosity for SF is almost the same with gases and 10 times less than liquids.

Instrumentation for supercritical fluid extraction (SFE) The necessary apparatus for a SFE Fig.1.setup are simple. It contains an extractor vessel, separators, CO_2 storage vessel, heaters and coolers. The mobile phase is usually CO_2. The method is fast. The lower viscosity of SF facilitates taking the component into the mobile phase. The solving power of SF can be promoted by tuning temperature or pressure.

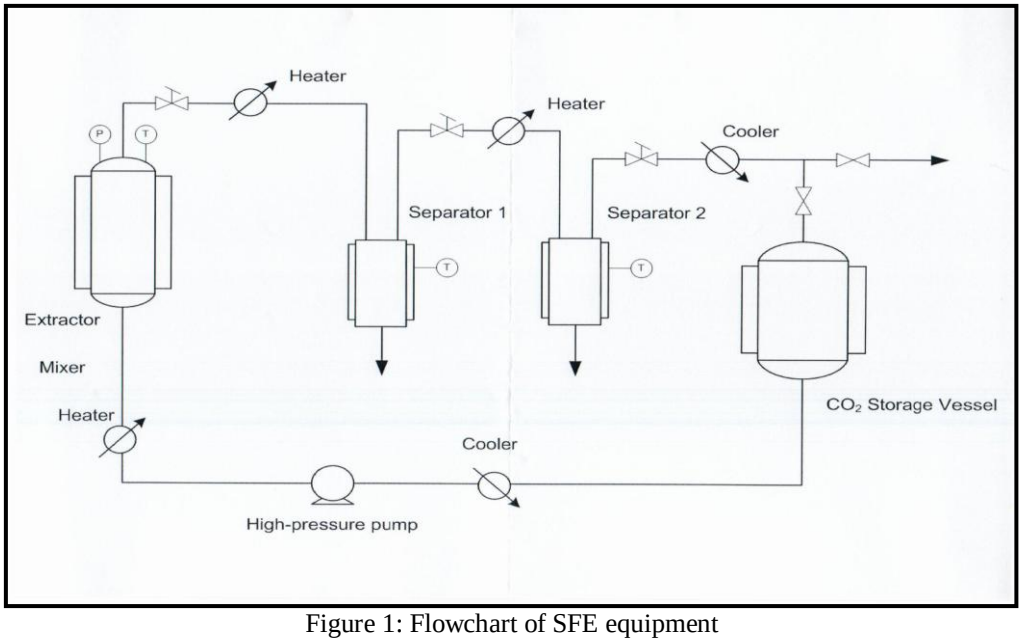


Figure 1: Flowchart of SFE equipment

The basic principle of SFE is that when the feed material is contacted with a supercritical fluid than the volatile substances will partition into the supercritical phase. After the dissolution of soluble material the supercritical fluid containing the dissolved substances is removed from the feed material. The extracted component is then completely separated from

the SCF by means of a temperature and/or pressure change. The SCF is then may be recompressed to the extraction conditions and recycled.

SFE can be applied to oils and fats, pesticides, organic pollutants, volatile toxins, polyaromatic hydrocarbons, cholesterol, pharmaceutical metabolites etc.

2. RESULTS AND DISCUSSION

Soxhlet extarctions were performed with nettle dried leaf base, using ethanol as solvent. The experimental data are collected in Table1.

Table 1: Soxhlet extraction data

Weight of dried nettle	Dry matter content of dried nettle	Dry matter content of extract	(Dmc of ext/Dmc of dn)*100	(Dmc of ext/Dmc of dn)*100
	Dmc of dn	Dmc of ext		average
gram	gram	gram	%	%
19,8977	18,0671	4,1380	22,904	
18,9128	17,1728	3,4491	20,085	21,05
20,2255	18,3648	4,0774	20,160	

The supercritical extraction experiments applied the same nettle dried leafs and supercritical CO_2 as solvent. Table 2. contains the experimental data.

Table 2: Supercritical extraction data

Measurements	Time	m_{CO2}	V_{CO2}	Dry matter content of extract		Used CO_2
	min	kg	m^3	kg	%	kg CO_2/kg dry matter
			21,195			
1.	26	2,855	22,426	$1,92*10^{-3}$	0,418	6,207
2.	30	3,120	23,980	$1,67*10^{-3}$	0,363	6,782
3.	26	2,975	25,320	$1,06*10^{-3}$	0,230	6,468
4.	35	4,063	27,254	$0,8*10^{-3}$	0,175	8,832
5.	35	4,068	29,194	$0,69*10^{-3}$	0,150	8.844
6.	20	1,948	31,273	$0,52*10^{-3}$	0,113	8,658
7.	17	2,035	34,098	$0,32*10^{-3}$	0,069	11.792
						21,195
						58,142

The Fig.2. shows the increase of amount of extract depending on increasing of CO_2. The same quantity of CO_2 can solve increasingly less extract from nettle drug.

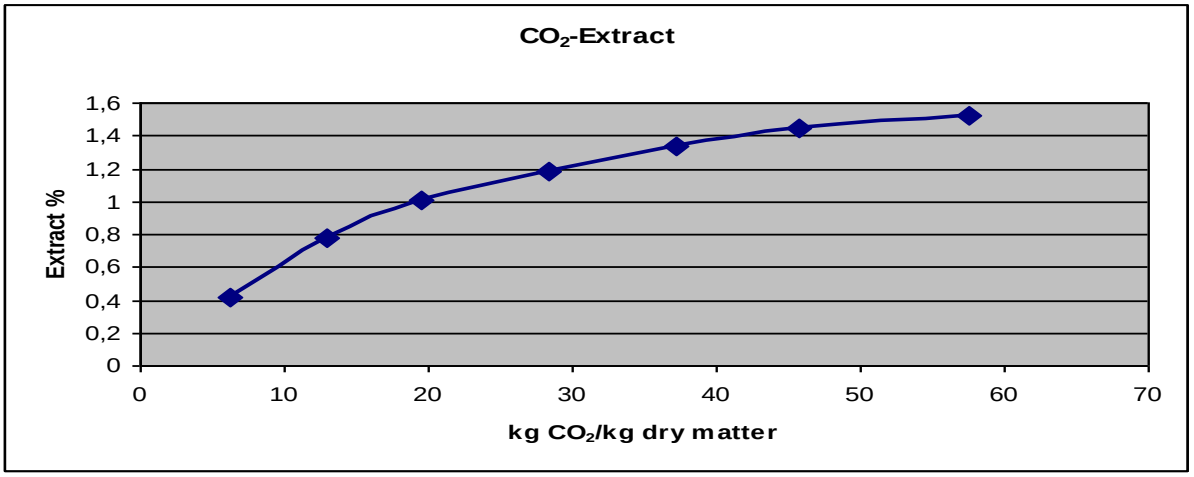


Figure 2: Process of extraction

As biologically active chemicals the total polyphenolic content and the total antioxidants were analized by spectrophotometer. In the first case the FOLIN method, in the second case the FRAP and ARTS-TEAC methods were applied.

3. CONCLUSIONS AND RECOMMENDATIONS

Table 3: Comparison the results of Soxhlet extraction and SFE

	Extractant	T	P	Dried nettle	Dry matter content of dried nettle	Dmc of extr.	(Dmc of ext/Dmc of dn)*100
		C°	bar	average, gram	average, gram	average, gram	average, %
SOXHLET	96% ETOH	105	1	19,679	17,868	3,888	21,05
SFE	CO_2 solid	40	450	506,3	459,72	6,98	1,518

After these first experiments we intend to continue the measurements using both extraction methods. In case of Soxhlet extraction the influence of solvent concentration on the yield of valuable components will be measured. For the investigation of the supercritical extraction, the influence of extraction pressure and temperature will be the changed independent parameters and a 2^n type experimental design will be performed to see the change of the yield of valuable components.

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EFFECT OF WATER SUPPLY ON INGREDIENTS WITH HUMAN NUTRITION IMPORTANCE IN TOMATO

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SUMMARY

In the frame of a R+D project entitled „Development of high added value vegetable-based product lines and processing technologies in the interest of the healthy nutrition – USOK2009" ingredients such as lycopene, polyphenols, flavonoid and vitamin C with human nutrition importance of different tomato varieties produced under different cultivation techniques including water supply were measured between 2009 and 2013. Values of the measured parameters were influenced by rainfall detected by an appropriate equipment of the given year. As a general rule it can be said that either in table tomato varieties or in industrial ones that increasing artificial water supply caused decreased levels vitamin C, polyphenols, quercetin and lycopene, as well. Studies were supported by USOK2009 project (TECH_09-A3-2009-0230).

THE BIO YEAST EFFECT OF THE POLYPHENOLIC COMPOSITION OF WINES

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SUMMARY

Due to the growing organic grapes, organic wine is a growing market for wine yeast species known as bio-dealers marketed yeasts. Present study focuses on the impact of so-called organic yeast such on the market in the wines. Control samples inoculated conventional yeast for wine. In addition to the basic analysis in fermented wine polyphenol composition was measured. Measurement results are evaluated by mathematical and statistical methods. Overall ratings present experimental results that there is no significant difference between samples, the bio yeasts did not affect the composition of the wine polyphenols.

1. INTRODUCTION

Polyphenols and the color agents which included contitute the most imortant compounds group. Collocation from PERI and POMPEI (1971) distinguish between non-flavonoid phenols, flavonoids, phenols and tannins. These compounds are transferred to the wine from the grapes. Their concentration is greatly affected by including the method of cultivation technology, variety, ripeness, year effect.

Prone to oxidation, cause for barnulással and other spin-offs and their presence is extremely important in shaping the nature in redwines (EPERJESI et. al., 2000). Flavonoid phenols play a major role in red wine flavor and aroma training, in contrast the non-flavonoid phenols are the larger group of phenolic for white wines (LŐRINCZ et. al., 1998). The flavonoid concretation of white wines are most of catechins (flav-3-ol) and leukoantociacnins (3,4 diol) (CARO et. al., 2010). However, these compounds carry a bitter taste impression and their presence is only a limited amount of desirable. The wines may increase the catchy taste due to the wooden barrels, as then hydrolysable tannins soulabe in the wine. The cinnamic aldehyde and benzaldehyde derivatives which extracted from oak barrels also contribute to the bitter sense of taste from the non-flavonoid phenols (KÁLLAY, NYITRAINÉ-SÁRDY, 2008). Also the Aprox. 25 mg/l average amount of tyrosol may contribute to the bitter taste sensation of white wines. Tyrosine formed by oxidative decarboxyl during the alcoholic fermentation (RIBEREAU-GAYON et.al., 2000). On the other hand increases the antioxidant content and the free radical scavenging capacity of white wines.

The bio yeast selected and dried propagated biological conditions. The soil used to the multiplication is comes from biological certified cultivation. Preparation of organic wines produced from grapes require special care, not only in the field of production, but also the entire economy cellar too.

Our experiments focused on the study of wine polyphenolic composition. In our measurements we examined whether there is a polyphenolic composition difference between the organic inoculated and the yeast has been used vaccinated wines.

2. MATERIALS AND METHODS

The experiments were performed using the wineyard of Nyakashegy Ltd. The samples were prepared in three replicates with model solutions. The harvest date was on 2012.09.05. The initial brix 20,6MM0 and the initial titratable acidity 6,2 g/l. The fresh grape juice was loaded into 2 liter sized ballons. The bio samples were engrafted with 20 g/hl bio yeast and the applause solution were added both middle and end of the fermentation.

Test methods
chemicals required

- Formic acid (AR spectral quality)
- Methanol (HPLC grade)
- tirozol (p-hydroxifenilethanol) standard (Sigma)

Equipment:	HP 1090 HPLC
Detection:	HP, diode-line
Column:	Nucleosil 100-5C18 5µ(4,0x250 mm)
Flow:	4 ml/min.
T°C:	40
λ:	280 nm
Eluent composition:	A: 0,5 v/v% aqueous formic acid solution
	B: 2,0 v/v% formic acid-methanol solution

The total polyphenolics content was measured using the method with Folin-Ciocalteu phenol reagent and expressed as gallic acid equivalent for your reference (KÁLLAY, TÖRÖK, 1999).

The leukoantocianins content which spectrophotometrically determination based on the modified method by FLANZY (1970) using a heated 40:60 mixture of the HCl-butanol and iron (II) sulfate. The anthocyanin content measured at 550 nm spectrophotometer in 96% ethanol dilutions containing 2 v / v% HCl concentration also under FLANZY's (1970) modified method. The catechin content in diluted alcohol wine vanilinnel reacted with sulfuric acid, 500 nm spectrophotometrically (REBELEIN, 1965).

3. RESULTS AND DISCUSSIONS

The main results of the first analysis are summarized in Table 1. Measurement results are the average of three-fold repetition of the table. Our results show that there is no significant difference between samples except the alcohol content and residual sugar concentration. It is obvious that the fermentation process normally run in the control sample, i.e., the residual sugar content of only 2 g / l. However, the organic yeast was partially able to fermented the sugar, namely 63.4 g / l of sugar remains in the wine. The wine technology technology is an important issue in the amount of volatile acidity. The present studies show that the volatile acid well below of the critical value (1 g / l). Glycerol content also was in line with the literature, it is known that glycerol - pyruvic acid fermentation occurs the glycerol in the early stages of alcoholic fermentation, an average of 6-10 g / l concentration

Table 1.: Analytical results

	SO_2 (mg/l)	titratable acidity (g/l)	pH
controll	46/105	7,1	3,36
organic yeast	55/140	7,0	3,31

	alcohol (v/v%)	sugar (g/l)	volatile acid (g/l)	glycerin (g/l)
controll	13,89	2,2	0,37	6,16
organic yeast	9,77	3,4	0,59	6,53

Measurements of the polyphenol composition was evaluated by Student's t-test. The results in the chart triple repetition of the mean values.

Measurement results show that there is no significant difference between the samples with 95% significance level. The total polyphenol content in both samples showed almost the same value. We were unable to detect significant differences between the samples of catechin concentration in the case of leukoantocianin content either. No significant difference was found between the samples simple phenolic composition and concentration of tyrosol.

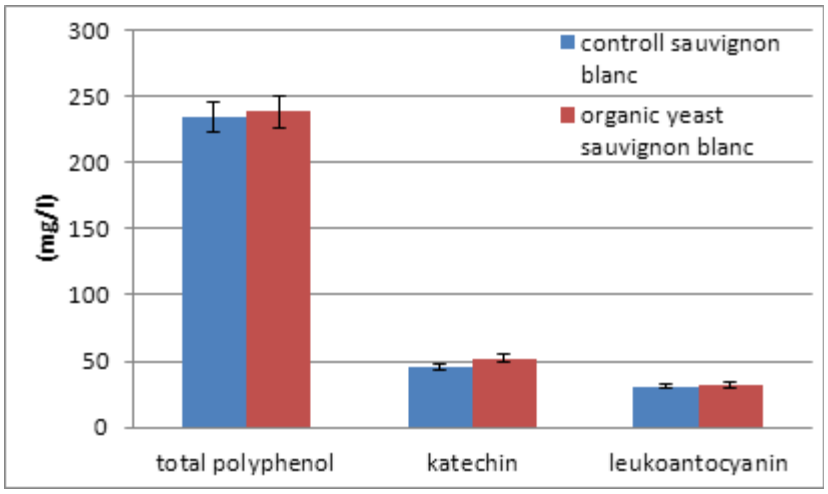

Figure 1: The polyphenol composition of the samples

The overall conclusion is that the experimental conditions for organic yeast did not change the polyphenolic composition of samples and the and simple phenolic composition. Our measurements and experiments were continued and extended to wines made from grape varieties resistant from organic viticulture which is a good basis for forming.

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ESTIMATION OF OVERALL MASS TRANSFER COEFFICIENT IN OSMOTIC DISTILLATION FOR GENTLE SOUR CHERRY JUICE CONCENTRATION

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SUMMARY

Different salt solutions such as CH_3CO_2K, NH_4NO_3, K_2CO_3, and $CaCl_2$ have been examined as alternatives for osmotic distillation process concentrating pre-concentrated sour cherry juice. Simplified estimation of mass transfer phenomena has been also performed. Further analytical methods such as total antioxidant activity, and total polyphenolic content using spectrophotometric assays have been also carried out to evaluate the effect of the osmotic distillation treatments on valuable compounds losses of concentrated sour cherry juice.

1. INRODUCTION

Thermal concentration techniques such as evaporation or vacuum evaporation can result subsequent losses of valuable compounds such as aromatic compounds, vitamins or other components with health care benefits. Osmotic distillation (OD) is an emerging alternative for concentration of thermosensible solutions such as fruit juices (Valdés, 2009). In this process the hydrophobic nature of a porous membrane prevents penetration of the pores by aqueous solutions, creating air gaps within the membrane. The difference of water activity (i.e. vapour pressure) in between the two sides of the membrane induces a component transport across the membrane pores: vapour is transferred from the higher vapour pressure phase to the lower one. This transfer can take place at isothermal conditions thus driving force has to be generated by using an osmotic agent (OA) with sufficiently low vapour pressure.

Selection of an appropriate OA for use on an industrial scale should be in accordance with several requirements. It has to be thermally stable, non-volatile. Also, the OA has to be non-toxic, food-grade quality and should also be readily available at low cost (Shin, 2007). Furthermore the salt should have a high osmotic activity (high water-solubility and low equivalent weight) in order to maintain a low water vapour pressure above the solution and, thereby, maximize the driving force for water removal. Finally, the salt solution should ideally be noncorrosive to ferrous alloys, such as stainless steel.

The aim of this short study is to explore new opportunities in OD treatment using alternative solutions as OA. Saturated CH_3CO_2K solution is studied and compared with traditional $CaCl_2$ solution and other, and previously not examined salt solutions such as NH_4NO_3, and K_2CO_3. Pre-concentrated sour cherry juice is concentrated further from 20°Brix to 60°Brix using all the examined OA and the performance of these processes is calculated and compared. Simplified estimations of the overall mass transfer coefficient of the most effective OD solution and the classical $CaCl_2$ solution are also performed. Finally, total antioxidant activity (TAA), and total polyphenolic content (TPC) of the sour cherry juice are determined before and after the treatments and losses of TAA and TPC during the OD processes are calculated.

2. MATERIALS AND METHODS

2.1. Sour cherry juice concetration experiments

Mixture of five cultivars of sour cherries ("Érdi bőtermő", "Debreceni bőtermő", Oblacsinszka", "Pipacs I", "Újfehértói fürtös") is used for the experiments. Initial total soluble solid (TSS) of juice is 9.2 °Brix. After pressing, sour cherry juice is clarified using cross-flow microfiltration. Tubular ceramic membrane is applied with 0.45 µm pore size (Millipore, Billerica, MA, USA) at 25°C and 1.5 bar transmembrane pressure difference. Furthermore reverse osmosis is applied to pre-concentrate the juice using spiral wound module (MFT Köln, CD1TYP3, Köln, Germany) with 98% of NaCl retention at 30°C and 30 bar transmembrane pressure difference. 20°Brix of TSS is reached. Saturated salt solutions are prepared at 30°C using deionized water and crystal form of salts (VWR International Ltd, Debrecen, Hungary). Table 1 illustrates the saturation solubility of the salts at 30°C.

Table 1: Saturation solubility of osmotic agents at 30°C

Osmotic agent	Saturation concentration, g/100 g water
CH_3CO_2K	283.8
NH_4NO_3	241.8
K_2CO_3	113.7
$CaCl_2$	102

Source: J.H. Perry (1968): Chemical Engineers Handbook, Műszaki kiadó, Budapest.

MD 020 CP 2N hollow fiber polypropylene porous membrane with 0.2 µm pore size is applied for OD experiments and the useful area of this membrane is 0.2 m^2 (Microdyn-Nadir GmbH, Wiesbaden, Germany). Feed stream circulates in the capillaries and the OA streams circulate in the shell side of the module in counter-flow mode with 20 L h^{-1} recirculating flow rate. All the tests are carried out in batch mode. The initial amount of sour cherry juice is 800 g and the initial masses of OAs are 2000 g in all cases. Membrane module is heat-isolated and heat exchangers are applied to keep stagnant the adjusted 30°C operation temperature.

The calculation of performance is based on increase of the mass of osmotic agent during process and is recorded by a digital balance connected to a PC. Equation 1 defines the calculation process performance as permeate flux:

$$J = \frac{1}{A}\frac{\Delta m}{\Delta t} \quad (1)$$

2.3. Simplified calculation of overall mass transfer coefficient

Equation 2 describes the permeate flux during OD process based on the mass transport phenomenon. If water activity of the feed and osmotic solution streams can be determined, a simple function can be used to calculate vapour pressure above a certain composition of feed or osmotic agent stream. It is illustrated in Equation 3. Based on the previous equations (Eq. 1-3), K can be estimated using Equation 4:

$$J = K\Delta P_b \quad (2)$$

$$P_b = a_{w,b}P_w^0 \quad (3)$$

$$K = \frac{J}{P_w^0 \Delta a_{w,b}} \quad (4)$$

Water activity values of the feed sour cherry juice and the two most effective osmotic agents are determined using Novasina LabMASTER-a_w (Novasina AG, Lachen, Switzerland) equipment. Figure 1 illustrates the Water activity values of sour cherry juice, $CaCl_2$ and CH_3CO_2K in function of total soluble solids.

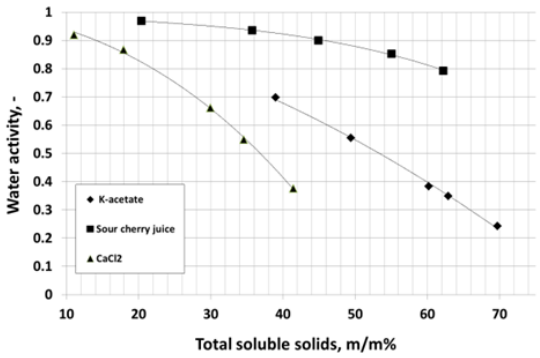

Figure 1: Water activity of sour cherry juice, $CaCl_2$ and CH_3CO_2K in function of total soluble solids (30°C).

2.4. Analytical measurement of sour cherry juice concentration

For determining antioxidant activity, spectrophotometric FRAP assay (Ferric Reducing Ability of Plasma) is applied (Benzie, 1996). The antioxidant power was calculated from a standard curve obtained by different concentrations of ascorbic acid. Total amount of soluble phenols were determined using Folin-Ciocalteu's reagent according to the method of Singleton (Singleton, 1965). The content of soluble phenols was calculated from a standard curve obtained by different concentrations of gallic acid.

3. RESULTS AND DISCUSSIONS

3.1. Results of the osmotic distillation experiments

Figure 2a illustrates the results of the OD operations. It is well observed that CH_3CO_2K was found to be the most effective and it was followed by the $CaCl_2$ solution. NH_4NO_3, K_2CO_3 are less effective than the previously mentioned. It has to be remarked that the difference in between CH_3CO_2K and $CaCl_2$ is less pronounced the last part of the process.

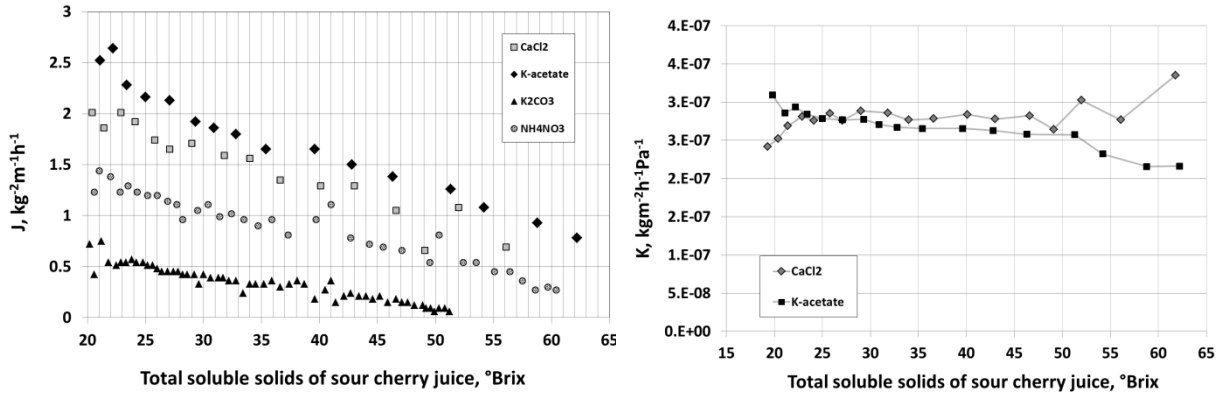

Figure 2: a. Permeate flux during the concentration of sour cherry juice using different osmotic agents (left), b. overall mass transfer coefficient in of CH_3CO_2K and $CaCl_2$ in function of TSS os sour cherry juice (right).

On Figure 2b is also well observed that during the concentration process the "effectiveness" of the CH_3CO_2K is decreasing in contrasts the $CaCl_2$. This phenomenon can be explained by the differences of the physical chemical properties of the solutions such as diffusivity and viscosity – however to be sure on this presumption further measurements are required.

3.2. Results of the analytical measurement of sour cherry juice concentration

Table 2: Effect of osmotic distillation treatment on total antioxidant activity and polyphenolic content

Treatment	Op. time, min	AA, mMol AAE/L	TPC, mMol GAE/L
Initial juice	0	5.31 (SD=0.19)	6.41 (SD=0.25)
CH_3CO_2K	107	4.39 (SD=0.12)	4.56 (SD=0.18)
$CaCl_2$	120	4.36 (SD=0.12)	5.08 (SD=0.29)
NH_4NO_3	180	3.60 (SD=0.11)	4.25 (SD=0.18)
K_2CO_3	474	2.85 (SD=0.12)	3.50 (SD=0.16)

Table 2 shows the effect of the sour cherry juice concentration using osmotic distillation treatment applying different OAs. Robust Games-Howell statistical analysis was performed on the data. With significant level of α=0.05, it was found significant decreasing with the AA and TPC during the OD treatments.

4. CONCLUSIONS

However CH_3CO_2K was found to be the most effective OA, there was found significant losses of AA and TPC of the treatment. It can be an effect of the temperature, but because the system was not covered totally from the light, light cause such a loss.

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NOMENCLATURE

J - permeate flux, $kgm^{-2}h^{-1}$
A - useful membrane area, m^2
Δm - mass changing during a certain time, kg
Δt - time interval, s.
K - overall mass transfer coefficient, $kgm^{-2}h^{-1}Pa^{-1}$
ΔP_b - vapour pressure difference in between the two stream bulk phase, Pa
P_b - vapor pressure above the bulk phase of feed and osmotic agent stream, Pa
a_{wb} - water activity of bulk phase of the feed and osmotic agent stream, 1;
P_w^0 - pure water vapour pressure at certain temperature, Pa. (T=30°C, P_w^0 = 4243 Pa)

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EXAMINATION OF WHEY DEGREASING WITH COMBINED MEMBRANE FILTRATION

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SUMMARY

The largest quantities of by-product of the dairy, namely whey comes from the cheese making. The whey proteins are used by the agriculture in animal nutrition, and by the human nutrition as well; dry soups, infant formulas and supplements. The aim of our experiments was the separation of the lipid fraction of whey. During the measurments 0.05 µm, 0.2 µm and 0.45 µm microfiltration membranes were used in vibrating membrane filtration equipment (VSEP) and in a laboratory tubular membrane module. During the microfiltration, analytical characteristics, the fouling and the retention values were examined. Using the VSEP and the tubular module made possible to compare the effect of vibration, the static mixer and/ the airflow on the separation parameters.

1. INTRODUCTION

Liquid whey contains lactose, vitamins, protein, and minerals, along with traces of fat. During the degreasing cream can be skimmed from whey. Whey cream is more salty, tangy, and “cheesy” than ("sweet") cream skimmed from milk, and can be used to make whey butter. The other reason of the degreasing is the further processing of the whey for dry powder/nutritional supplement. The membrane degreasing methods are new ones and the biggest gap of it is the low flux and high resistances. These effects could be mitigated by used membrane modes of us, i.e. static mixer, aeration and vibrating.

Newtonian fluids such as an aqueous solution, - are being turbulent flow in most industrial applications, but within a short pipe section this turbulence is not enough to equalize temperature or concentration in-homogeneities. The use of static stirrers was made better amalgamation than increase the speed or the pressure during the process. The flux is increased and the operating cost is decreased at tubular membranes with static mixer (Krstic et al. 2002). The fouling of the membranes was possible to decrease at the introduction of gas into the liquid (Laboire et al. 1998, Cabassud et al. 2001, Cui and Wright, 1996). The introduction of a specific gas - in this case air - directly into the fluid created a two-phase gas/liquid flow. The efficiency of the separation is influenced by the position of the membrane (vertical or horizontal) and the direction of the flow (up or down). The aeration method is limited by the gas distribution and the management of this process (Derradji, 2000). During the vibratory shear enhanced process (VSEP), the filtering parameters (flux, retention and resistances) were investigated by the effects of the vibration. This is another solution to decrease fouling (Frappart and trs, 2008). The shears strengths at the surface of the membrane can be increased by vary the frequency of the vibratory membrane module. The polarization layer, the resistance values, and the fouling were measured by the effect of vibration, and the evolution of retention values were measured by the effect of increase of vibrational amplitude (Ahmadu et al. 2009, Hodúr et al. 2009).

2. MATERIALS AND METHODS

Sweet cheese whey was used for measurement which came from Soma Budapest Ltd. Its basic analytical parameters are: fat content: 0.18 m/m%, protein content: 0.33 m/m%, milk sugar content: 2.61 m/m%, dry materials: 3.72 m/m%, total protein content: 0.47 m/m%. The degreasing process was made by membrane separation. These basic parameters were measured by Bentley milk analyzer equipment.

The air injection and/or static steering method were implemented by tubular and hollow fiber membranes with 0.45, 0.2 microns, 0.05 microns cut off value. The tubular membrane was 250 mm length, and it was included 1 tube which has an internal diameter of 7 mm.. The applied static mixer was a 250 mm lenght Helix type metal static stirrer with a pitch of 0,006 m and an inner radius of the stirrer of 0.0035 m. (KenicsTM, Helix).. The KenicsTM type static stirrer (made by plastic material) was used also with a length of 241 mm, and a thickness of 1 mm, a diameter of 6.35 mm. The flux was performed on 100 L/h recirculation flowrate, on 0.2 MPa transmembrane pressures and on 20 L/h air injection rate. In all measurments the initial amount of feed was 2 L of sweet whey. The temperature was a permanent 30°C degree during the tests. The airflow was introduced into the fluid flow before the membrane module.

Vibratory filtration equipment set marketed by New Logic International Corp. and this equipment was used at L-mode (L: laboratory methods: the module comprises one disk-shaped membrane with an active filter surface 503cm2). The VSEP system consists of disk-shaped flat-sheet membranes. This laboratory module attached to a central shaft. The shaft was rotated a short distance at a frequency of 54 Hz. 0.2 μm cut-off values membranes (made of polyethersulfone) were used during the measurements, on a transmembrane pressure at 0.3 MPa. In this equipment the initial amount of feed was 10 L of sweet whey.

The samples were taken at different intervals during the measurement from retentate and also from permeate.

3. RESULTS AND DISCUSSION

The tubular membranes were used during the measurement at a pore size of 0.2 microns. The retention of the fat component was important in our research programme, and we were able to retain more than 50% at low pressure with using static mixer.

The flux values were measured at 0.2 MPa transmembrane pressure, and at 100 L/h recirculation flow rate by a 0.45 μm tubular membrane. The flux values are started at 60 L/m^2h. The flux values were showed 17-18 L/m^2h values during the normal filtration process, and with combined the air injection, the flux was decreased slight by the air flow on a 0.2 μm membrane. When the *Helix* static mixer element was used in the filtration process, the flux values were increased two times greater extent, from 18 L/m^2h to 40 L/m^2h when the *Helix* static mixer was used with air injection.

The increase of the flux is holding until the 0.2 MPa transmembrane pressures; because on higher pressure values this increase of the flux is started to show a strong deceleration (1. Figure). When the air injection process was used alone, the flux values remained very low; therefore the air injection method itself is not a recommended method for whey processing. When the *Helix* static mixer was used alone in the equipment under the same parameters, the flux values were showed higher values than the experiments with air injection, but above 0.2 MPa transmembrane pressures values, the flux was strongly decreased, therefore it was justifed to use the lower transmembrane pressure.

The 0.2 microns membrane was used with *Kenics* type static mixer in second period of our research programmes. The flux values (J = 45 L/m^2h) were increased by the *Kenics* static

mixer, but the increasing was not as high as using the *Helix*-type static mixer (J = 53 L/m^{2}h). Our experiments were continued by a 0.05 microns pore size tubular membrane to comparing the received data with the other tubular membranes different data. The flux was increased 50-80% during the filtration process by using a 0.05 microns pore size tubular membrane with a *Kinetics* static mixer. The flux was decreased strongly after 0.3 MPa transmembrane pressures. The vibratory shear enhanced membrane filtration was examined by a 0.2 µm pore size microfiltration membrane, on 0.3 MPa transmembrane pressures with and without using vibration (54 Hz).

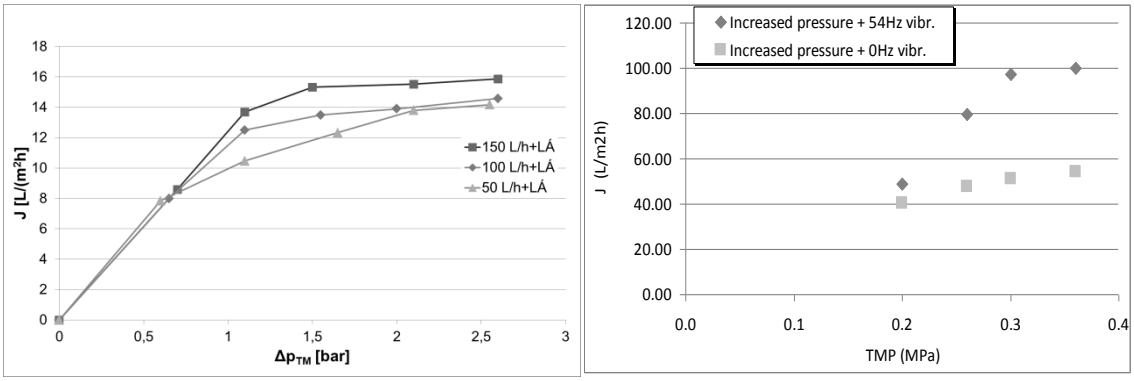

Figure 1: The changes of whey flux (J) as a function of trasnmembrane pressure – a) at different recirculation flow rate (LÁ: air injection, HelixSK: Helix type static stirrer) and b) vibrated and non vibrated methods

The retention values were measured only from the fat molecules. The examination of the resistance values was showed that the gel layer and the membrane resistance values showed the same magnitude values. The fouling resistance was showed an order of magnitude lower value than the two other determinative resistance values before.

In non-vibrating mode, not only the total resistance value was showed differences, but its structure and distribution as well. Without using vibration during the separation process, the flux values were showed four times lower; the total resistance value was showed one order of magnitude higher; and the fouling resistance values were showed two orders of magnitude higher values.

The drag resistance values were decreased by the vibration, therefore this change was allowed the fat molecules to move and accumulate on the membrane surface (2. Figure) The flexible fat molecules were moved into the capillaries of the membrane under pressure and without vibration, where due to their sizes (3.5 microns), these molecules were fouled inside the membrane capillaries. The increased retention values and their absolute magnitude were been significant by fat content. The retention values of the small components were increased by the fouled pores.

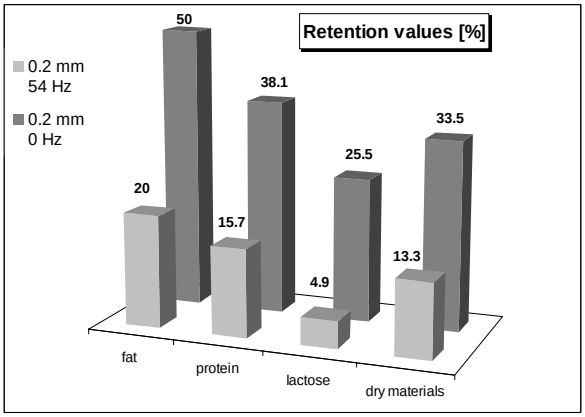

Figure : The retention value of most important components of whey

4. CONCLUSION

The experiences showed that the 0.45 microns pore size membrane could slightly hold back the fat molecules, due to their larger pore size. The desired filtration results were achieved by the measurements of 0.2 MPa and 100 L/h.

The 45 % higher flux values were measured by Helix static stirrer against the normal filtration process, but the combination of the static stirrer and the air injection were made the highest flux values (30 % higher than the filtration process by the Helix static stirrer) under the same conditions. Comparing the two different static stirrers, it was found, that the 15 % higher flux values were measured by Helix static stirrer than the Kenics stirrer. This means that the separation of the fat content was easier and more effective by using the combination of static stirrer and air injection.

The vibratory shear enhanced process was showed that not only the retention values of the fat content were increased without vibration, but the other elements retention values too. 300 % higher flux values were measured by 54 Hz vibration than without vibration.

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BIOGAS PRODUCTION IN DAIRY WASTE WATER

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SUMMARY
The dairy industry produced in large amount of waste water, which contains organic matter, therefore the treatment of this water is necessary. Nowadays, the physical and biological treatment cleaning methods are preferred instead of chemical methods. During these experiments the biological purification of dairy waste water and biogas producing ability by using anaerobic microorganism culture was investigated. The results showed that the preparation and the addition of microorganism culture have a major effect on the quality and time course of the biogas producing. Biogas production of different concentration of model dairy waste water were tested and compared to a model dairy waste water ultrafiltration (UF) concentrate. It was found that the biogas yield of UF concentrate was favourable.

1. INTRODUCTION

Food industry companies operating in the sectors that are stated below can economically produce hydrogen and biogas from their wastes: distilleries and bioethanol plants, breweries, sugar mills, meat processing factories, veterinaries and sanitation plants, starch and treacle production plants, yeast production plants, milk-processing plants, bakeries, chips and potato processing plants, juice and tinned food producers, wineries and fish processing plants. These mentioned food industries are very suitable for such an application (Coskun 2012). The dairy industry is one of the main sources of industrial effluent in the world. This industry is based on the processing and manufacturing of raw milk into products such as yogurt, ice cream, butter, cheese and various types of desserts by means of different processes, such as pasteurization, coagulation, filtration, centrifugation, chilling, etc.. The characteristics of dairy effluents may vary significantly, depending on the final products, system type and operation methods used in the manufacturing plant. These wastewaters are mainly composed by different dilutions of milk (or transformed products), and washing water containing alkaline and acidic chemicals after the cleaning of bottles, tanks, process equipment (tools, pumps) (Carvalho 2013). Among available technologies for wastewater treatment, membrane technology used in wide range. Ultrafiltration (UF) of dairy wastewater yields a high permeate flux at low transmembrane pressure. The permeate water can be recycled in the plant, and the concentrated retentate can be used as feed supplement for animals, as substrates for biosynthesis of biodegradable plastics or for anaerobic digestion to produce hydrogen (Luo 2011). Anaerobic degradation is a technology which combines the treatment of residues or wastewaters with the production of methane, a renewable energy resource with least greenhouse emissions as compared with fuel alone (Murphy 2006, Berglund 2006). Therefore biogas production by utilizing anaerobic digestion processes is a good alternative renewable energy source (Coskun 2012, Sundh 2003). Anaerobic digestion is a process by which almost any organic waste can be biologically converted in the absence of oxygen. This is a complex process, which requires specific environmental conditions and different bacterial populations. Mixed bacterial populations degrade organic compounds, thus producing, as end-product, a valuable high energy mixture of gases (mainly CH_4 and CO_2) termed biogas. By means of enzymes produced by different kinds of bacteria, the organic waste undergo as the following three main reactions. In hydrolysis the organic macromolecules, i.e. carbohydrates, proteins and fats, are de-polymerised by extracellular enzymes. The produced monomers undergo degrading reactions, which produce acetic acid, long chain fatty acids and CO_2. In the acetic acid formation step different bacteria, degrading long chain fatty acids, later produce acetic

acid, molecular hydrogen and CO_2. Acetic acid can even be produced from the CO_2 and H_2 and from fatty acids, alcohols and carbohydrates. The final step is the production of methane. Acetic acid is finally degraded with methane production by the so-called methanogenic bacteria. These bacteria are highly sensitive to the O_2 concentration in the system. Their inactivity depends on an increasing fatty and acetic acids concentration within the environment, consequently lowering pH, whose measure, in a well-balanced system, has to range between 7 and 8 (Lastella 2002).

The aim of our work was tocompare model dairy waste water, and ultrafiltration treated dairy waste water retentate biogas production. The effect of inoculum concentration in synthetic water during anaerobic digestion was investigated and the optimal parameters were determined.

2. MATERIALS AND METHODS

Model solutions were prepared from milk powder, concentrations was 0.3 wt.%. The synthetic waste water contain glucose 8 g/L, aluminium hydrogen carbonate 0.4 g/L, potassium dihydrogen phosphate 0.4 g/L, sodium hydrogen carbonate 0.4 g/L and trce metal solution A and B in each 1 ml/L concentrate. Trace metal solution A made from $MgSO_4 \times 7H_2O$ 5 g/L and solution B made from $FeCl_3$ 5 g/L, $CaCl_2$ 5 g/L, KCl 5 g/L, $CoCl_2$ 1 g/L and $NiCl_2$ 1 g/L (Anaerobic Digester's Instruction manual 2009).

Flat-sheet Regenerated cellulose membranes (C-10F series, New Logic, USA) (MWCO 10 kDa) were used. The membrane effective area was 0.004534 m^2. For the anaerobic digestion Microcat-UASB microorganism culture (Biosience Inc.) was used. For the CH_4 determination 45% KOH solution was used prepared from analytical grade solid KOH (Ferrokémia KTSZ).

Experimental design:

The UF experiments were carried out in batch stirred ultrafiltration cell (Millipore, Serial N°96, USA) with a capacity of 50 cm^3, which continuous stirred at 50 rpm. The initial feed volume was 50 cm^3. The UF experiments were carried out until 40 cm^3 of the total sample was filtered.

Batch digestion was carried out in triplicate using the Oxitop-C (WTW, Germany) system, which had a total nominal volume of 250 cm^3. 100 cm^3 sample of different concentration Microcat-UASB inoculum was added to the reactor. The pH was checked with a Consort multiparameter analyser before and after the digestion. The initial pH was between 7.0 and 7.2. The temperature was kept at (35 ±1)°C in Thermostat cabinet (Lovibond) (Nadais 2001).

In the experiment the pressure was monitored at the reactors, in the KOH solution absorbed the CO_2. Over the 24 day digestion period, 411 pressure readings were taken i.e. once every 84 min. Pressure was released when above 300 hPa. Pressure data were converted to volumes of gas using the ideal gas law according to Eq. (1)

$$V_a = \frac{P_h V_h}{P_a} \quad (1)$$

where *V* is volume of biogas at 35 °C, *P* is pressure and subscripts *a* and *h* are atmosphere and headspace volume, respectively (Devlin 2011).

In the preparation of inoculum at first step shake a one pound sample of dry microcat bran-based product, than made 5g/L solution with warmed (27-28°C) distiled water and mix for 0.5 hour.

3. RESULTS AND DISCUSSION

In the first series of experiments the inoculum concentrate was determined with a 0,8 g / 100 cm^3 glucose containing synthetic waste water. Digestion speed and produced biogas and methane volume were compared at different concentrates (1%, 3% and 5%) of inoculum The results showed (Fig 1a), that the 5% inoculum concentrate resulted the fast gas producing, but the cumulated gas production (Fig 1b) was very similar to the 3% inoculum concentrate at pH 7.02. Therefore in the next series of experiment 3% of inoculum concentrate was applied.

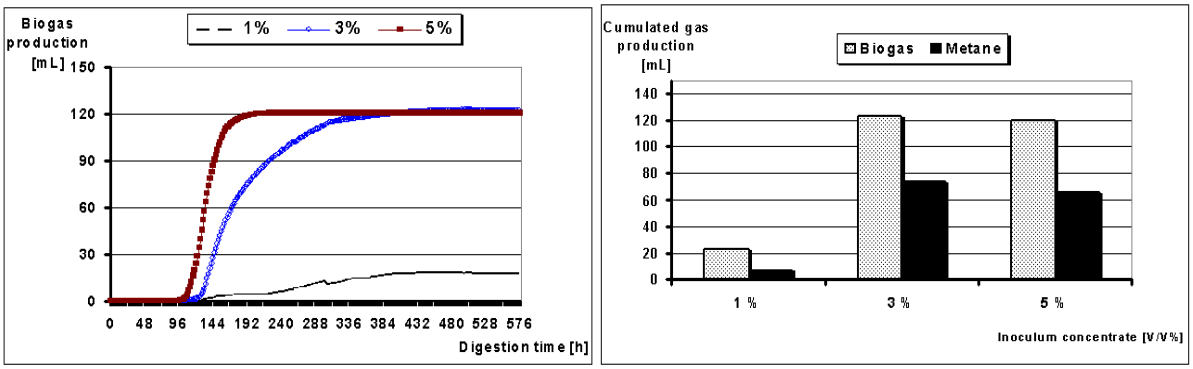

Figure 1: (a) biogas production vs digestion time, (b) Cumulated gas production

In the next series of experiments anerobic digestion of model dairy waste waters made from different concentration (0.3% and 0.6%) of milk powder were examined. It was found that anaerobic digestion of ultrafiltration retentate of 0.3% dairy waste water resulted the fast and highest methane production and very similar results were achieved during digestion of the 0.3% dairy waste water (Fig 2a). The cumulated biogas productions were lower (Fig 2b), than the first series of experiment, because the average pH decreased at 6.68, and the dairy waste water content was different as the synthetic waste water.

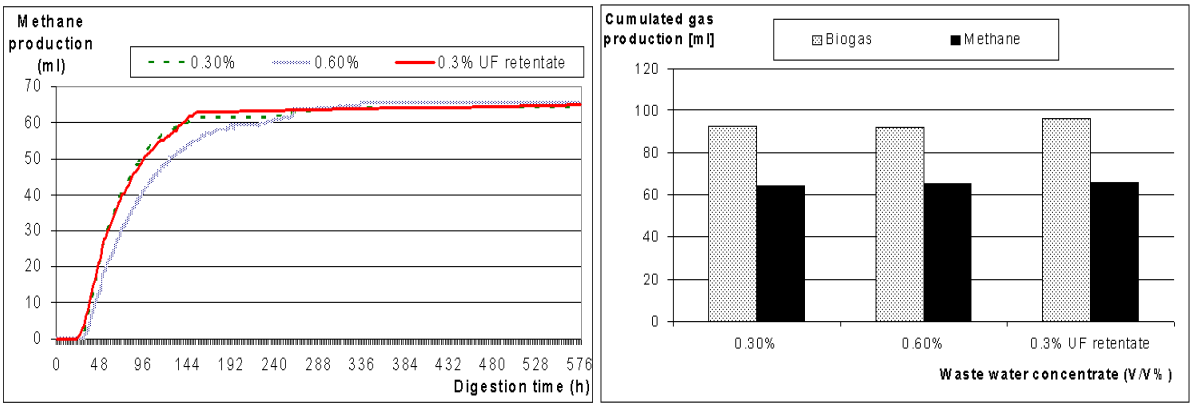

Figure 2: (a) methane production vs digestion time, (b) cumulated gas production

4. CONCLUSIONS

In this work biogas production of model dairy waste water was investigated. The preliminary studies showed that the fast and highest methane production was achieved at 3% inoculum concentrate. The biogas production from dairy waste waters strongly depends on pH, the ultrafiltration retentate and the feed produced very similar volume of methane.

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Anaerobic Digester's (W8) Instruction manual, Armfield Issue 12 (2009) pp. 43

PRODUCTION OF MICROEMULSION BY CERAMIC TUBE MEMBRANE EQUIPPED WITH STATIC TURBULENCE PROMOTER

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SUMMARY
Membrane emulsification (ME) is a relatively new technique for the highly controlled production of particulates. In this process, the dispersed phase (oil) is pressed through the pores of a microporous membrane directly into the continuous phase (water) flowing tangentially to the membrane surface. The purpose of the emulsification experimentations was to find and model operating conditions of the operation. In laboratory experiments from conventional, commercially grade sunflower oil (dispersed phase) and from distilled water (solid phase) emulsions were prepared. The ceramic tube membrane with nominal pore size of 1.4μm was used in the experiments (ZrO2). In order to increase the shear-stress near the membrane wall (influence the characteristics of the flow regime of the continuous phase), a kind of self-fabricated helical-shaped-ribbon reducer was installed inside the tube membrane.

1. INTRODUCTION

This paper deals with the highly controlled production of particulates. In the course of membrane emulsification, the dispersed phase (oil) is pressed through the pores of a microporous membrane directly into the continuous phase (water) flowing tangentially to the membrane surface. Emulsified droplets are formed and detached at the end of the pores with a drop-by-drop mechanism (De Luca et al., 2008). In order to ensure a regular droplet detachment from the pore outlets, shear stress is generated at the membrane/continuous phase interface by recirculating the continuous phase using a pump or by agitation in a stirring vessel (Vladisavljevic, Williams, 2005).

One of the innovative methods for generating shear at the surface of a membrane for the purpose of ME is by pulsing the flow over the membrane surface formed from a tube (Richard G. H. et al., 2010, 2013). In literature it is reported that helical baffles are likely to perform better compared to rod inserts, implying that the helical vortices improve the mixing between the boundary layer on the surface of the membrane and the bulk fluid to a greater degree than by simply generating turbulent flow using cylindrical rod inserted (Ahmad et al., 2005).

According to these researches Koris et al. established that, with a simple mechanical method, by insertion of static turbulence promoters, it was possible to increase shear stress at the membrane surface, while maintaining a low shear in the recirculation loop (Koris et al., 2011). The present paper deals with a simple, static method, which is cost-effective and is able to increase the productivity of cross-flow membrane emulsification with satisfying quality.

2. MATERIALS AND METHODS

2.1. Materials and membrane

In laboratory experiments from conventional, commercially grade sunflower oil (dispersed phase) and from distilled water solution (continuous phase) emulsions were prepared. The 1.5 m/m % continuous phase was formed from Tween 80 (Sigma-Aldrich Chemie GmbH) organic surfactant and distilled water.

The ceramic tube membrane (Pall Schumacher Corporation) with nominal pore size of 1.4 μm was used in the experiments (active ZrO_2 layer). The droplet size and the droplet size

distribution in the emulsion samples made with this membrane were measured by Fritsch Laser Particle Sizer Analysette 22.

2.2. Apparatus

A cross flow membrane emulsification system was used to produce all emulsions. The experimental set-up used for the experiments is shown in Fig. 1. The apparatus is very simple; it was designed from practical application viewpoint. It included two manometers, one placed before membrane and the other placed after the membrane, were used to measure the pressure drop through the membrane. The continuous phase was recirculated on the lumen side of the membrane by a submersible pump. The oil pressure was guaranteed by compressed air with an air pump and it was injected from the outer surface of the membrane.

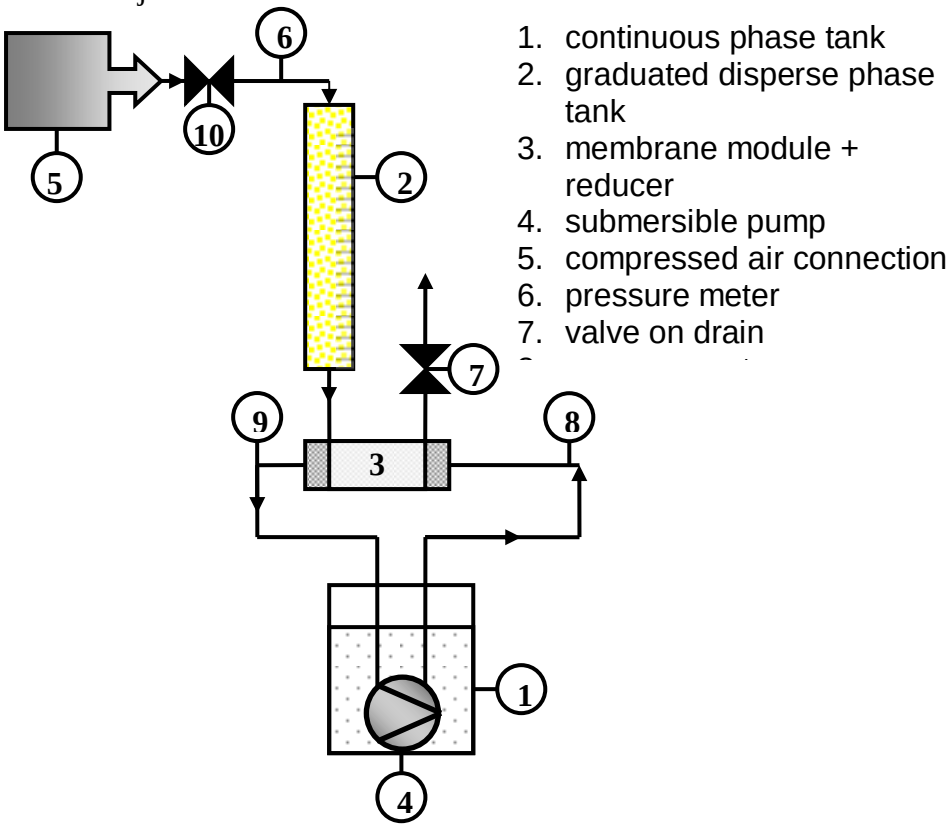

Figure 1: Scheme of the cross-flow membrane emulsification apparatus

2.3. Membrane emulsification and experimental design

In the emulsification experiments two parameters were changed: the pressure of disperse phase (expressed in DF-driving force) and the recirculation flow (shear stress at the wall of the membrane).The disperse phase flux was determined by volume, upon the oil consumption from the graduated feed tube by using formulas. The experiments were carried out at room temperature. The axial cross-flow velocity in the tube membranes was varied by 0.35 m/s and 0.7 m/s when the re-circulated flow-rate (RFR) was set to 50 L/hr and 100 L/hr respectively on the pump. The shear-stress was observed as 0.4 Pa and 0.8 Pa accordingly. The emulsion was also prepared without any cross-flow in which case the re-circulated flow rate and shear stress were 0. The applied transmembrane pressure (TMP) was varied between 1 and 1.8 bars.

The system and the membrane were cleaned between the experiments with ULTRASIL-11 (~1% m/m) solution when it was necessary. In order to increase the shear-stress near the

membrane wall (influence the characteristics of the flow regime of the continuous phase), two kind of self-fabricated helical-shaped-ribbon reducers were installed inside the tube membrane.

Figure 2.: Simulated figure of the helix reducers

To indicate the results statistical methods, 3^p type full factorial experimental designs were evaluated, using software called STATISTICA. In this experiment, the effect of driving force (DF), shear stress (τ) (regulated by flow rate) and pore size were investigated as independent variables. On the contrary, the fluxes, diameter of droplets and span were used as dependent variables. The effects of independent variables on dependent variables were observed.

3. RESULTS AND DISCUSSION

3.1. Response surface model for the effects of driving force and shear-stress on (a) flux, (b) droplet size and (c) span

The effects of driving force and shear-stress above the membrane surface can be seen in the following graphs in case of empty tube (Fig.3a-c), in case of using reducer 1 (Fig.4a-c) and in case of using reducer 2 (Fig.5a-c).

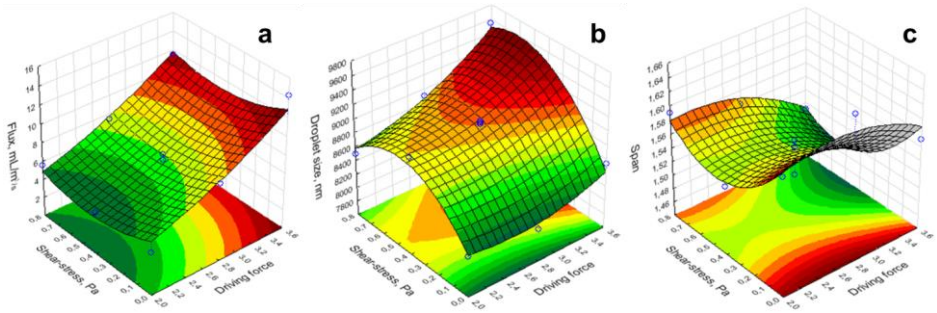

Figure 3: Fitted 3D surfaces the effects of driving force and shear-stress on (a) flux, (b) droplet size and (c) span in case of empty tube, 1.4µm

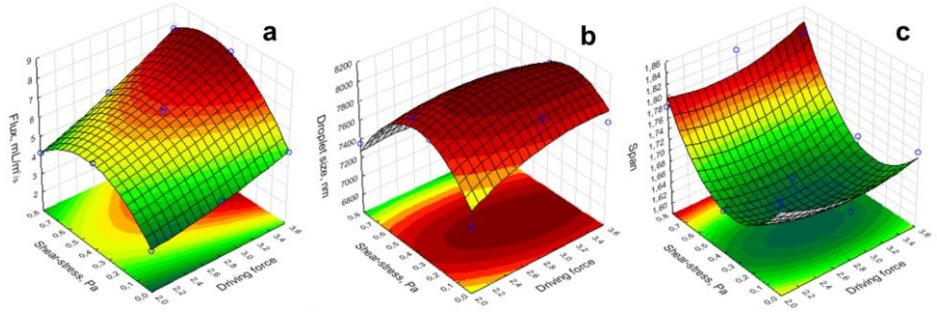

Figure 4: Fitted 3D surfaces the effects of driving force and shear-stress on (a) flux, (b) droplet size and (c) span in case of using reducer 1, 1.4µm

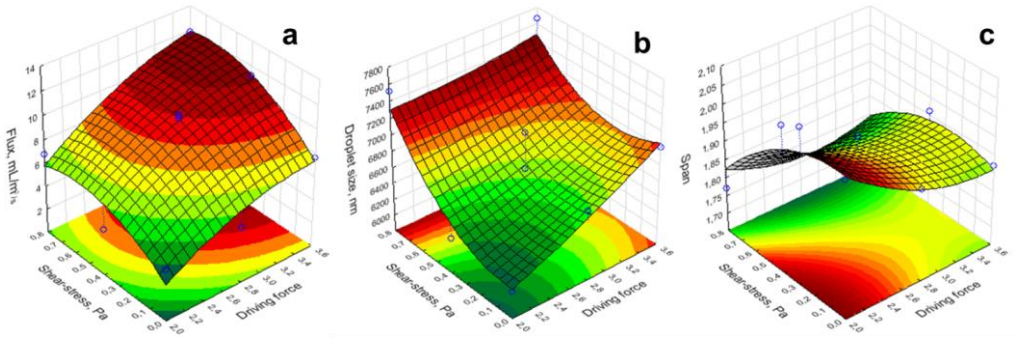

Figure.5: Fitted 3D surfaces the effects of driving force and shear-stress on (a) flux, (b) droplet size and (c) span in case of using reducer 2, 1.4µm

4. CONCLUSIONS

Fine quality emulsions were obtained with low span values when monodisperse O/W emulsions were prepared using commercial available microfiltration tubular ceramic membranes. The response surface methodology proved to be adequate modeling tool for mathematical representation of the process. For prediction of the flux, droplet size and span a mathematical model was set up which can describe well the dependent variables in the studied range, namely the run of the flux and the mean droplet diameter and the effects of operating parameters. The application of helical-shaped reducer has positive effects on emulsification process, when the turbulence promoter is inserted fluid flow pattern are changed. In most of the cases around 8% less energy required for recirculation of the continuous phase and emulsion with reducer are comparable to the case when no reducer was used.

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MONITORING THE QUALITY CHANGES OF POTATO DURING STORAGE BY MEANS OF DIFFUSE REFLECTION METHOD

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SUMMARY

The key question of the food supply chain, including logistics, is the behavior of quality in time. This information is essential in postharvest technology processes from field to fork, and also in the prediction of shelf-life. Potato tubers were analyzed during the presented project using diffuse reflection (optical method), acoustic firmness and dynamic impact measurements. The experiment lasted one month with two measurements in a week. The obtained data showed high variability, however relationship was found between daily averages of optical parameters and dynamic firmness values. The results are promising and encourage further development of a quality prediction system based on optical measurements.

1. INTRODUCTION

One of the most important postharvest quality parameters of vegetables is the firmness. This property plays an important role during storage and in the decision making process for the consumer as well. Due to the numerous advantages of non-destructive methods, their role is increasing more and more. Two non-destructive firmness measurement methods (the acoustic response technique and the impact method) were used successfully in many researches. According to Felföldi and Fekete (2000) the acoustic method gives relevant information about the overall stiffness of the sample, and the impact method characterizes the firmness of the sample surface. By the help of these methods, the firmness related quality changes of fruits and vegetables can be detected. Molina-Delgado and co-workers (2009) found that the measurements based on impact method are highly sensitive to the changes in turgidity of apple and suitable for the detection of differences between apple fruits from different harvest dates. Both acoustic and impact methods were found to be capable of sensing firmness loss as induced by storage circumstances in case of apple and tomato (De Ketelaere et al., 2006).

The diffusion of light is a physical phenomenon which depends on the cell size, intra-, and extracellular properties of tissue. The appearing laser halo on the crop's surface is recorded by a vision system, after processing of the obtained images we can get information about the texture and mechanical properties of the crop (Lu, 2004). Prediction of the soluble solids content and firmness of apple was based on hyperspectral image data in the work of Quing (2007). Changes of optical properties and firmness of apple during drying were characterized by multispectral data (Romano et al., 2011). Monte Carlo simulation was used to follow photon packages in kiwifruits and apple tissue in order to build inverse models for the processing of diffuse reflection images (Baranyai and Zude, 2008, 2009). Qin and Lu (2009) used Monte Carlo models to simulate light transport in apple tissue and they performed measurements with hyperspectral diffuse reflectance imaging system at the wavelength range of 500 to 1000 nm. The light penetration depth into the fruit tissue was highly influenced by pigments and moisture content.

Objective of the presented work was to monitor the changes of the stiffness during storage by non-contact methods.

2. MATERIALS AND METHODS

2.1. Materials

The amount of 110 pieces of commercial potato were stored at room temperature for one month. Randomly selected tubers were withdrawn for measurement twice or three times per week.

2.2. Optical method

A diffuse reflection imaging system was assembled for this research. The device was equipped with a high performance monochromatic CMOS camera (Photon Focus MV1-D1312 Gigabit Ethernet Series, gray scale resolution of 12 bit) with an L-SV-L5014MP megapixel lens of fixed focus, optimized for visual and near infrared applications. Seven solid-state laser diode modules emitting at different spectral bands (635, 650, 780, 808, 850 and 1064 nm) were used. All laser light beams were adjusted to 15° incident angle in order to inject laser beam into the potato tissue with minimal direct reflection. This illuminated area was scanned and intensity values were calculated with radial averaging relative to the incident point. Threshold of the 50 % intensity level was used to segment illuminated area and facilitate automatic detection of incident point. The location of the incident point was identified by weighted average. Full width at half maximum (FWHM), distance of inflection point (DIP) and slope of the logarithmic intensity profile (SLP) were calculated.

2.3. Non- destructive methods

The textural changes were measured by acoustic impulse response technique and impact method. Acoustic impulse response technique was described by the firmness coefficient. For the calculation of the mentioned coefficient the characteristic frequency and the sample mass were used (Valente et al., 2009):

$$S = f \times m^{2/3} \quad (1)$$

where

S: acoustic stiffness coefficient, 10^6 Hz^2 $g^{2/3}$; f: characteristic frequency of the sample, Hz; m: sample mass, g.

Impact measurements were performed on similar points of the samples like the optical measurement. The measuring system consisted of an impact hammer with a changeable mass and a built-in piezoelectric accelerometer, an electronic signal converter and a dynamic signal analyzer recording and displaying the voltage signal of the accelerometer. Time and voltage differences between initial and maximum point of the curve were determined by a special program (developed by Physics and Control Department of Corvinus University of Budapest). The sample's firmness is characterized by the impact stiffness coefficient (Felföldi, 1996):

$$D = 1/\Delta T^2 \quad (2)$$

where

D - impact stiffness coefficient, ms^{-2}; ΔT - time difference between initial and maximum point of the curve, ms.

2.3. Statistical analysis

Raw data was collected in Microsoft Excel® 2003. The program R (2.13.2, R Foundation for Statistical Computing, Vienna, Austria) was applied for the visualization, the extraction of the selected parameters of intensity profiles and to accomplish the statistical evaluation.

3. RESULTS AND DISCUSSIONS

Measurements' data were averaged per day, because the variability of the samples was high. The samples with horticultural origin are usually characterized by this kind of variability.

3.1. Changes of the stiffness coefficients during storage

On Figure 1 the typical exponential change of the horticultural product's stiffness is shown. On the first plot the acoustic stiffness (S), on the second the impact stiffness (D) is presented. With both methods the changes of the stiffness were well traceable. The process of softening was described by exponential models. The equations of the models are at the top of the plots. The two fitted models are acceptable according to the determination coefficients (R^2=0.984 and 0.979) and the relatively low RMSEP values (2.19 $\times 10^5$ Hz^2 $g^{2/3}$; 8 $\times 10^{-3}$ ms^{-2}). Based on the Durbin-Watson test, the models do not contain systematic error. In case of the measurement of D, the stiffness decreased to approximately the one-third part compared to the initial value; however the S decreased only to the half part of the initial value.

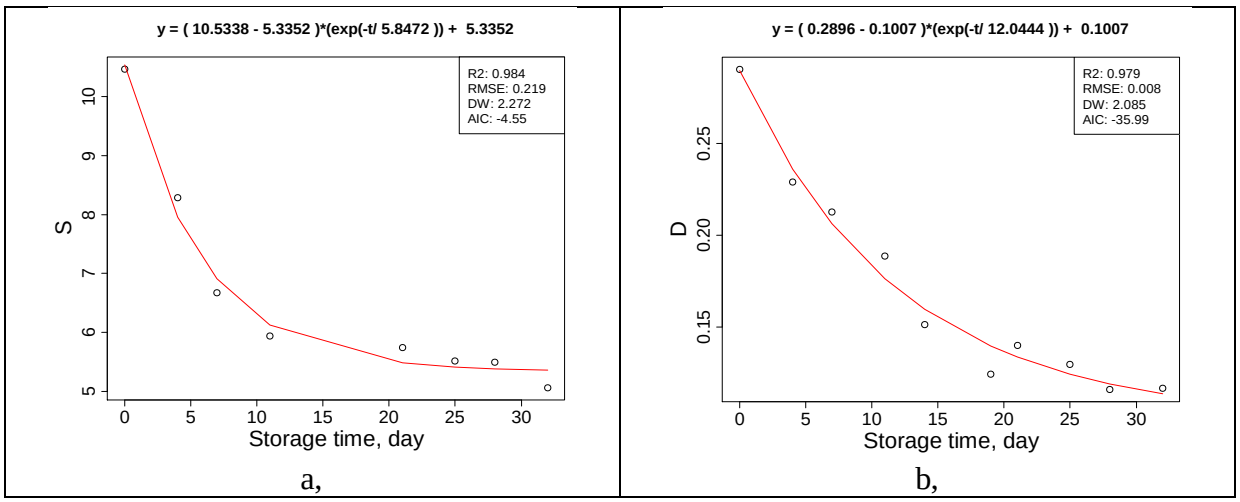

Figure 1: Changes of the acoustic (a) and impact (b) stiffness values during storage

3.2. Changes of the optical parameters during storage

Besides the measurement of the stiffness coefficients the changes during storage were monitored by diffuse reflection optical method as well. Measurements were performed by seven lasers and three special parameters (FWHM, DIP, SLP) were defined, thus 21 variables were obtained. For the statistical evaluation only the FWHM values acquired from the profiles of two selected wavelengths (635 and 650 nm) were applied. The selections were based on the error bars. On Figure 2 the results based on the selected two lasers' parameters are shown. The two fitted models are following an exponential-like tendency.

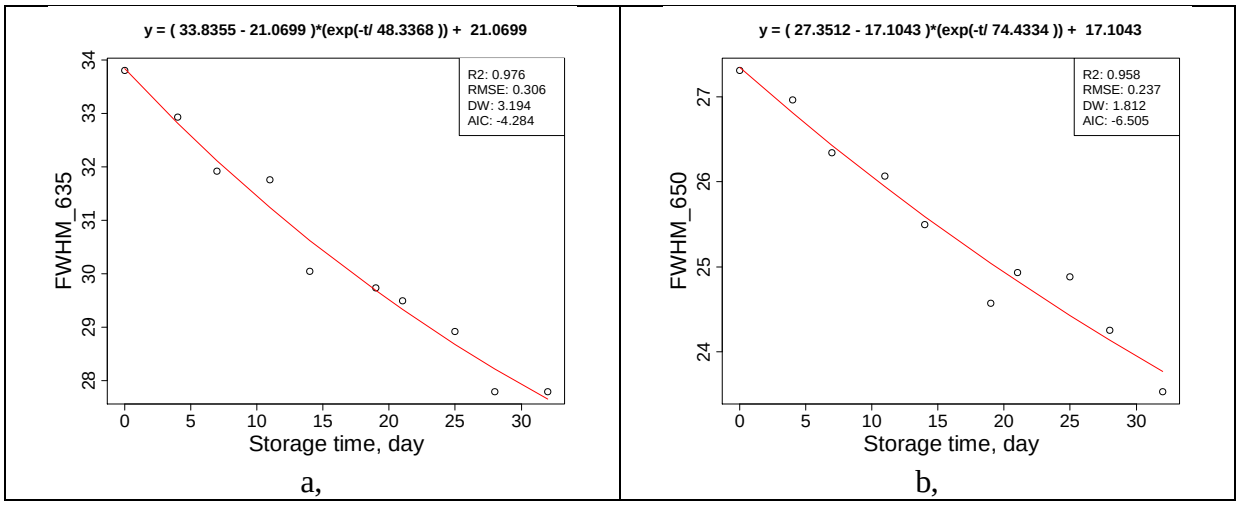

Figure 2: Scattering parameters changing with the storage time

3.3. PLS prediction by diffuse reflection parameters

Multivariate method (PLS with leave-one-out cross validation) was used to build prediction models for the non- destructive quality parameter (D) and storage time (shelf-life). The two predicted models are acceptable according to the determination coefficients (R^2=0.96 and 0.97) and the relatively low RMSEP values ($RMSEP_{lgD}$=6 10^{-2} ms^{-2}; $RMSEP_{day}$=1.63 day) Based on the Durbin-Watson test the models do not contain systematic error.

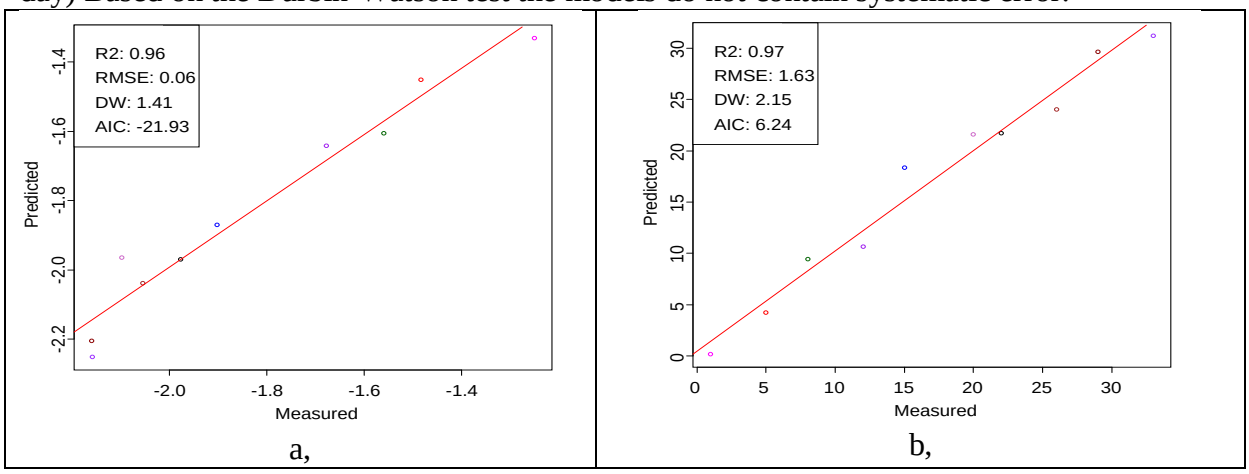

Figure 3: PLS prediction of lg D (a) and storage day (b) by laser scattering parameters

4. CONCLUSIONS

Both acoustic and impact firmness methods were found to be able to follow stiffness decay during the experiment. Effective PLS models were built for stiffness (impact) and shelf-life prediction based on diffuse reflection imaging. Results are promising and indicate the feasibility of a non-contact measurement technique. Although, statistical parameters shown that more robust models should be built on experiment of larger scale.

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OPTIMIZATION OF ELECTRONIC NOSE PARAMETERS IN CASE OF BERRY PUREES TREATED WITH COMBINED PRESERVATION TECHNIQUES

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SUMMARY
Electronic nose offers a fast and non-destructive way to sense aroma, so it can be effectually used to identify the numerous volatile compounds of foods. Nowadays, beside the challenges in food authenticity assessment, food quality control and shelf-life investigation, „e-nose" can be also applied to evaluate food freshness and to reveal changes caused by some preservation techniques. Berries, a group of fruits highly rich in aromas and bioactive components, apart from being consumed freshly, are also processed into purées, juices etc. However, conventional thermal processing technologies used for the inactivation of microorganisms and enzymes in these products generally involve higher temperatures, thus seriously affecting quality. Quality changes are not only related to the nutritional components, but impairment of colour, flavour and texture can be also observed. As consumers' demand for minimally processed and fresh food products increases, new preserving techniques, such as the high hydrostatic pressure (HHP) technology, become more and more popular. The objective of this study was to examine the volatile compounds of some berry purées treated with HHP and mild heat – both separately and in combination – by using electronic nose, and to optimize the e-nose parameters to improve the technique's efficiency when discriminating the groups of treated samples from each other.

1. INTRODUCTION

Human sensory panel is still one of the most effective and prevalent methods when analysis of objective attributes of food is required. Nevertheless, it also has drawbacks, like the subjectivity or the necessity of a highly qualified staff. It is therefore not surprising, that new scientific tools are already on their way. One of these instrumental achievements is the „electronic nose", which allows to acquire information about the relevant features of samples – in a similar way to that of an experienced taster using his perception of smell –, but eases the measuring process with its simplicity and rapidity.

During the past decades, the conception of electronic nose has gone through a huge advancement. The history of the artificial olfaction technology started with the development of the first gas multisensor array in 1982 (Persaud & Dodd, 1982). From that time advances in aroma-sensor technology, electronics and artificial intelligence made it possible to develop devices capable of characterizing volatile aromas released from a multitude sources. Gardner and Bartlett (1994) defined the electronic nose as a complex system that contains an electric chemosensor array and a data processing unit, capable of recognizing different „figures". When using the „e-nose", an intrinsical comparison is made between the actual chemosensor responses and the previous measurements. Each sensor's response is compared to the reference gas, the sequence and the distinction of the responses jointly result a unique footstep that can be used to identify a scent. Unlike other analytical instruments, these devices allow the identification of aroma mixtures as a whole without having to identify individual chemical compounds within the mixture. Getting proper information about the responses is only feasible through multivariate mathematical-statistical ways.

The purpose of this study was to determine the effects of high hydrostatic pressure (HHP) and heat on the volatiles of some berries by using electronic nose. In the first section, authors tried to explore the e-nose's signals in general – using control and treated strawberry samples –while in the second part – beside a sensory analysis –, an optimization process was carried out to improve the instrument's efficiency regarding the classification of berry purées treated with different techniques.

2. MATERIALS AND METHODS

Strawberry purées were used as raw samples, which had been portioned into small plastic pouches and heat-sealed, then put in frozen storage. After thawing, pressurizing was carried out in a *Resato FPU-100-2000* type equipment and heat treatment was performed in a temperature controlled water bath. Following the heat treatment, samples were chilled in ice-water.

In the first section, five types of strawberry samples were made: 1. *control*; 2. *heat-treated* (at 60 °C for 15 min.); 3. *HHP-treated* (at 600 MPa for 5 min.); 4. *heat- and then HHP-treated*; 5. *HHP- and then heat-treated*. Control and treated purées were put into special glass vials of the electronic nose (*NST 3320, Applied Sensor Technology*) that were then closed by Teflon coated septum. E-nose consisted of 10 metal oxide semiconductor field effect transistor (MOS) sensors, 12 metal oxide semiconductor (MOS) sensors and a sensor for the detection of relative humidity. Five grams of sample were put into each vial. Measurements were carried out at stock settings of the e-nose. The sequences of the vials were allocated by using a random number generator. The instrument examined each vial in three repetitions.

In the second part – based upon the results of the first series of experiments – an optimization process of the e-nose was executed to improve efficiency of the technique.

Linear discriminant analysis (LDA) was used in this work to obtain classification rules for differentiation between berry samples based on electronic nose measurement data (*SPSS v.20*). LDA provides a classification model, characterized by a linear dependence of the classification scores with respect to the descriptors (groups defined previously), which maximize the ratio between-class variance and minimize the ratio of within-class variance. LDA assumes an a priori knowledge of the group membership of each sample in a training set. The classification power of the model derived can be evaluated using the original grouped cases or using a 'leaving one-out' cross-validation (CV) procedure. In CV, the sample data minus one observation are used for the estimation of the discriminant functions, and then the omitted variable is classified from them. The procedure was repeated for all observations and so each sample was classified by discriminant functions which were estimated without its contribution. Sum of correctly classified samples is suitable for the determination of which LDA model has better separating ability.

3. RESULTS AND DISCUSSION

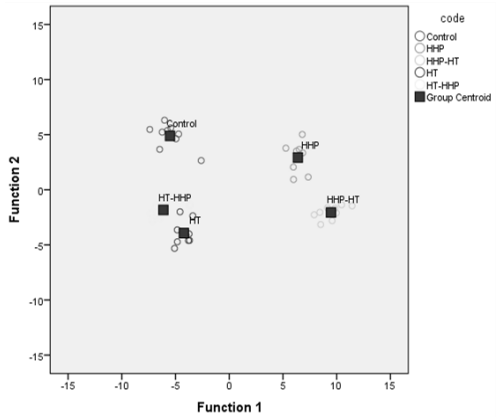

Figure 1. Discriminant analysis score plots of strawberry purées treated with different preserving techniques – based on the sensor-responses of electronic nose and using stock settings.

According to the results of the first section, sensors of the electronic nose proved to be appropriate to make distinctions between strawberry purées treated with different preserving techniques, although a bigger overlap appeared between the *heat-treated* (HT) and the *heat- then HHP-treated* (HT-HHP) groups (Fig 1.). Considering the outcomes of the cross-validation as well, the next section focused on the further examination of these two groups.

In the optimization process of the electronic nose settings, the following parameters and their effects on the discrimination of HT and HT-HHP groups were examined: amount of sample, incubation temperature and incubation time, sampling time, flushing time and regeneration time. Table 1. summarizes the important factors of the e-nose measurements, where the lower values represent the device's stock parameters. Numbers marked by *show those cases where better classification could be obtained. While increasing the amount of sample, the incubation temperature or the sampling time affected the efficiency of classification auspiciously, other parameters took no effect or worsened the results during cross-validation.

Table 1: Main factors and their effects on discrimination of different raspberry samples by electronic nose

	1	**2**	**3**	**4**	**5**	**6**	**% of correctly classified samples**
Amount of sample (g)	**5**	20	20	30	60	260	66.7
1	**7,5***						**88.9**
Incubation temperature (°C)	5	**20**	20	30	60	260	66.7
2		**40***					**77.8**
Incubation time (min.)	5	20	**20***	30	60	260	**66.7**
3			**30**				55.6
Sampling time (s)	5	20	20	**30**	60	260	66.7
4				**45***			**83.3**
Flushing time (s)	5	20	20	30	**60***	260	**66.7**
5					**90**		66.7
Regeneration time (s)	5	20	20	30	60	**260***	**66.7**
6						**520**	55.6

Application of parameter marked by * can separate groups more effective.

After defining proper parameters of e-nose measurement strawberry, raspberry and blackberry purées were compared. *Heat-treated* (HT) and *heat- then HHP-treated* (HT-HHP) samples were made to analyse the efficiency of classification of LDA model derived from different electronic nose measurements. Table 2. shows the percentage of correctly classified samples of discriminant analysis.

Table 2: Efficiency of discrimination of different berry samples by electronic nose

	% of correctly classified samples at cross-validation	
	stock settings	**optimised settings**
raspberry	68.8	**75***
strawberry	**93.8***	81.3
blackberry	**81.3***	56.3

Application of setting marked by * can separate groups more effective.

Results showed that the optimized settings of the electronic nose led to a better classification of raspberry purées, where the efficiency of classification increased during cross-validation. However, these effects were not so positive when measuring other berry samples. In these cases, the rate of miss-classification was far smaller when using stock settings.

4. CONLUSIONS

When summarizing the results, authors come to a quite peculiar consequence. Despite that the independently optimized parameters of the e-nose led to fairly better classifications in some cases, the collective effect of these sub-settings was no more so beneficial. That means, the standard settings of the device proved to be better in general.

The proper analysis of the results, the calculation of the Euclidean distances, showed that the optimized settings of the e-nose eventuated bigger sensor responses, but the difference between these responses was bigger, so thus the efficiency of classification was better at stock settings.

To sum up the experiences, electronic nose performed auspiciously when separated control samples from pressure and heat-treated ones, although results seemed to be dependent upon the type of berry. Nevertheless, the inspected phenomena associated with the settings of the e-nose could make a perfect ground for further investigations.

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EFFECT OF HIGH HYDROSTATIC PRESSURE TREATMENTS ON VOLATILES OF BERRY PUREES

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SUMMARY
High hydrostatic pressure (HHP) technology, as a promising alternative of thermal-treatment and chemical preservatives, can be used to produce minimally processed foods. It has the advantage of affecting only non-covalent bonds of macromolecules in foods and thus preserves nutritional components, taste and flavour exceptionally well. However, HHP also influences enzymatic reactions of food. Although some of these changes are often beneficial, monitoring the potential effects of high pressure treatments – especially in the field of product and technology development – is essential. To draw proper conclusions, there is an unequivocal need to integrate objective measurement tools into the generally non-objective sensory evaluations. Accordingly, it is useful and scientifically justified to apply „artificial sensory tools" when analysing the objective attributes of treated samples. The aim of this study was to point out some parameters of high hydrostatic pressure technique (pressure, temperature, build-up time, holding time, the number of cycles) that can substantially impact the sensory properties of treated products.

1. INTRODUCTION

Food-quality and food-safety are the most important factors influencing consumer choices, and are also primary factors for food manufacturers and distributors in modern times. High hydrostatic pressure (HHP) technology, as a non-thermal preserving technique capable of inactivating or eliminating pathogenic and food spoilage microorganisms while retaining the valuable components of foods, has the potential to serve both purposes simultaneously.

High hydrostatic pressure technology dates back over a century to the research of Hite (1899) – who performed experiments with a variety of foods at elevated pressures –, although the technology seemed to be unremarkable until the end of 1960's, when systematic researches started to explore its effects on microorganisms. Over the last 15-20 years, significant advances took place in the technology, mainly because high pressure equipment have become commercially available. Nowadays, HHP technique inspires dozens of manufacturers to produce innovative, natural-looking, fresh-like foods which satisfy consumers' needs.

When using HHP treatment, an elevated pressure is applied in an instantaneous way throughout the product and subjects foods to 100-1000 MPa under water or a special fluid as pressure transmitting medium. Due to the instantaneously transmitted pressure, processing time and conditions are independent of the volume and shape of the treated sample.

Besides the inactivation of microorganisms, there are some further effects of pressure on foods. Without the claim of completeness these are related to enzyme activation or inactivation, protein denaturation and modification, gel formation, not mentioning the changes in the properties of carbohydrates and fats.

Although it is generally assumed that the flavour of foods is not impaired by high pressure – since the structure of small volatile compounds is not affected –, HHP processing can induce some enzymatic and chemical reactions that finally cause changes in flavour, too.

The aim of this study was to determine some parameters of high hydrostatic pressure treatment that can significantly alter the volatiles of berry purées.

2. MATERIALS AND METHODS

Strawberry and raspberry purées were used as raw samples, which had been portioned into small plastic pouches and heat-sealed, then put in frozen storage. Following thawing, pressurizing was carried out in a *Resato FPU-100-2000* type equipment. Where higher temperatures were used, samples were chilled in icy water just after treatmentsTable 1. summarizes the parameters of each HHP treatment. The "build-up time" column means the interval while 100 MPa pressure-elevation was reached.

Table 1: Parameters of high hydrostatic pressure treatments

	Pressure (MPa)	**Temperature** (°C)	**Build-up time** (sec.)	**Holding time** (min.)	**Number of cycles** (pcs)
Treatment 1.	**400**	10	60	5	1 1 treatment cycle with a 5 min. – 300 sec. – long holding time
	500				
	600				
Treatment 2.	400	**10**	60	5	1 1 treatment cycle with a 5 min. – 300 s – long holding time
		30			
		50			
Treatment 3.	400	10	**60**	5	1 (1 treatment cycle with a 5 min. – 300 s – long holding time
			120		
			180		
Treatment 4.	400	10	60	**5**	1 1 treatment cycle with a 5 min. – 300 s – long holding time
				10	
				15	
Treatment 5.	400	10	60	5	1 1 treatment cycle with a 5 min. – 300 s – long holding time
					2 2 treatment cycles, each with a 2,5 min. – 150 s – long holding time
					3 3 treatment cycles, each with a 1,67 min. – 100 s – long holding time

Control (untreated) and treated purées were put into special glass vials of the electronic nose (*NST 3320, Applied Sensor Technology*) that were closed by Teflon coated septum.

E-nose consisted of 10 metal oxide semiconductor field effect transistor (MOS) sensors, 12 metal oxide semiconductor (MOS) sensors and a sensor for the detection of relative humidity. Five grams of sample were put into each vial. Measurements were carried out at stock settings of the e-nose (20 °C incubation temperature; 20 min. incubation time; 30 sec. sampling time; 60 sec. flushing time; 260 sec. regeneration time). The sequences of the vials were allocated by using a random number generator. The instrument examined each vial in three repetitions.

Linear discriminant analysis (LDA) was used to obtain classification rules for differentiation between berry samples when evaluating electronic nose measurement data (*SPSS v.20*).

Sensory analysis was performed by an untrained sensory panel, with 12 attendants in average, to compare the ability of the e-nose and human perception to differentiate between

control and treated berry purées. The panel evaluated the samples subjected to different HHP treatments by using a triangle test (MSZ ISO 6658-2001) where members were also asked to mark their judgements with a reliability index.

3. RESULTS AND DISCUSSION

Figure 1 and 2 show two typical examples of discriminant analysis results. They were calculated from sensor responses of electronic nose when different pressure levels (Fig 1) or different temperatures (Fig 2) were applied for treating raspberry purée samples. Based on the distance between different groups, similarity of groups can be evaluated. The closer the groups the less changes in volatiles. As it can be seen, control groups could be separated well from treated samples in both cases. Although the distances between treated samples were smaller than between control and treated samples, a clear trend could be observed in the location of treated sample groups. Application of higher treatment level resulted in higher distance from the control group.

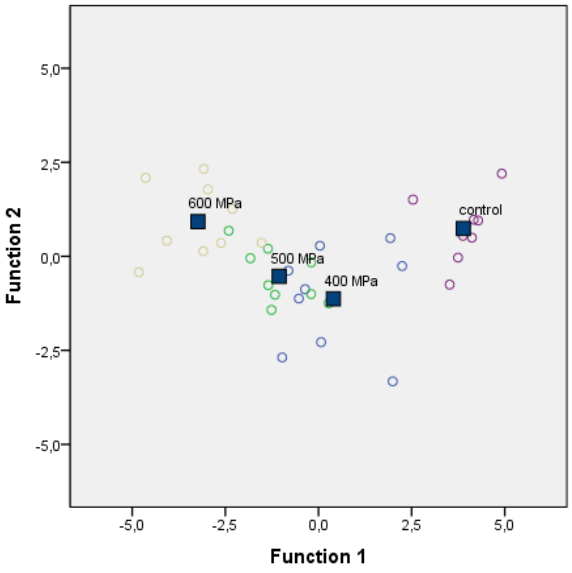

Figure 1: Discriminant analysis score plots of raspberry purées treated with high hydrostatic pressure treatments at *different pressure levels* – based on the sensor-responses of electronic nose

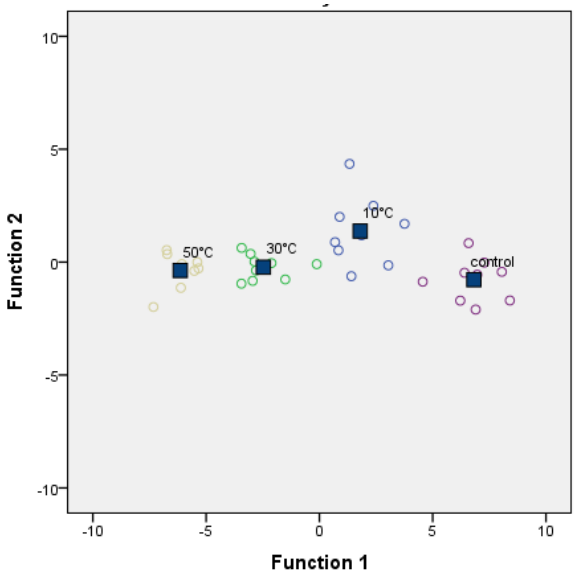

Figure 2: Discriminant analysis score plots of raspberry purées treated with high hydrostatic pressure treatments at *different temperatures* – based on the sensor-responses of electronic nose

Table 2. summarizes the main indices related to the efficiency of classification for both berry fruits. Eigenvalues are also represented in the table, which intrinsically compare the deviation measured between and within groups. The bigger the Eigenvalue is, the better the groups can be divided from each other. As it can be seen on Figures 1 and 2 the highest difference between groups is along the first function, so Table 2 contains the Eigenvalues of the first discriminant function only. Values marked by superscripts “a” or “b” show the cases of the best and the second-best classifications, respectively.

As Table 2. demonstrates, different holding times had the greatest effects on the discrimination of groups, and temperature had the second greatest. It can be seen that different pressures had minor effects regarding the discrimination of groups.

Results of the **sensory analysis** showed that attendants were not able to separate the control group from the treated samples in general. Even where correct answers were given, choices were not based on solid consideration.

Table 2: Effects of application of different HHP setting on the typical indices of discriminant analysis based on the sensor-responses of the electronic nose

Modified parameter of HHP treatment	Eigenvalue of the first discriminant function		Efficiency of classification (%)			
			Original model		Cross-validated	
	Raspberry	Strawberry	Raspberry	Strawberry	Raspberry	Strawberry
Pressure	7,554	0,922	88,9	66,7	58,3	33,3
Temperature	26,32	5,38[b]	**100[a]**	80,6[b]	**86,1[a]**	63,9[b]
Build-up time	28,957	3,302	97,2[b]	77,8	52,8	38,9
Holding time	54,109[b]	**24,221[a]**	97,2[b]	**100[a]**	**86,1[a]**	**72,2[a]**
Number of cycles	**55,256[a]**	1,445	94,4	69,4	75[b]	41,7

Superscript "a" and "b" mean the highest and the second-highest values relating to the best and the second-best classification.

4. CONLUSIONS

Results confirmed that the potential effects of high pressure treatment – combined with mild heat – on the volatiles of berry purées cannot be exactly prognosticated. For example, when samples were subjected to different pressures, treated groups of raspberry purées definitely separated from the control samples. At the same time, the difference diminished between strawberry groups where bigger overlaps appeared and the deviation of the individual samples within groups also increased.

Comparing the effects of the parameters, it could be stated that holding time and temperature principally affected the volatile compounds while the impact of different pressures was not so remarkable.

Furthermore, experiments confirmed that the human sensory panel couldn't differentiate between raw (control) and treated samples. Thus it could be conceived that e-nose proved to be a better tool when classifying control and HHP-treated berry purées.

ACKNOWLEDGEMENT: *The research of Valér Farkas grounded this publication was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4.A/1-11-1-2012-0001 „National Excellence Program". The equipment indispensable for making HHP treatments was founded by TÁMOP 4.2.1/B/09/1/KMR/-2010-0005 program of the National Development Agency.*

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MICROFILTRATION INTENSIFICATION (0,1µM) USING STATIC MIXER AND AERATION IN CASE OF WHEY FILTRATION

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SUMMARY
The effect of static mixer on whey microfiltration was studied using 0.1 µm pore size ceramic tube membrane. The aim of the experiments were to intensificate the membrane procedure by raising the flux, besides the retention of milk fat. During the experiment I studied the change of flux in case of changing the recirculation flow rate and the transmembrane pressure. I compared the pure water and natural whey fluxes in two cases: tube membrane + aeration and tube membrane + aeration + static mixer.

1. INTRODUCTION

Whey is a by-product of dairy industry. It mainly issues from the cheese and farm cheese production. Whey mostly used for animal feeding, or poured out as waste water. Whey contains a lot of valuable components, for example: whey protein, milk sugar, vitamin B – A – E. There are two different types of whey, called sweet and sour. They differ from each other in the way how they are prepared. There is a small difference in the percentage of their components, typically in pH, mineral components, lactose – and protein concentration. Whey is known for its positive effect on human health. We can use it as a cosmetology product or balsam (Homonnay and Koncz, 2005; Kohány, 2012).

2. MATERIALS AND METHODS

2.1. Materials

- distilled water
- whey (from Soma's Trade Kft, Budapest)
- P3 – Ultrasil – 11 (1m/V % soluted in distilled water)
- citric acid (1m/V % soluted in distilled water)

The typical composition of the sweet and sour whey can be found in Table 1.

Table 1: Distribution of whey components in general (sweet/sour) [Homonnay and Koncz, 2005.]

Components (g)	Whey (in 1000 ml)	Whey (in 1000 g)
Dry Matter	61	956
Milk Sugar	48/59	740/660
Protein	8	125
Fat	2	10
Lactic Acid	1/5	2/42
Mineral Component	5/7	80/105

2.2. Methods

Experiments were carried out using two different methods. In case of both cases 0,1 µm pore size ceramic tube membrane (details can be found in Table 2.) was used. In earlier experiments (Kohány, 2012.) made at the Department of Food Engineering different pore size (0,45 and 0,2 µm) microfiltration membrane were applied for whey filtration.

In these experiments inside the tubular membrane static mixer was applied (SM) in certain cases to lower the fouling phenomenon raising the flux values (using the equipment without static mixer: NSM). The lenght of the mixer was the same as the membrane 250 mm, helix type, with 0,006 m pitch, and inner radius 0,0035 m. Beside the static mixer also air-flow was applied to decrease the fouling. The flow rate of the air was constant: 20 L/h.
After the whey filtration P3 – Ultrasil solution was used for cleaning the membrane. The duration of the cleaning was 2 hours. Study shows that 1% P3 – Ultrasil can give flux recovery of about 80% (Popovic et al., 2009).

Table 2: The parameters of the applied membrane

Type	T1-70-20-Z
Producer	Membralox
Material	ZrO_2
Chanel Diameter	7 mm
Lenght	250 mm
Surface Area Filtration	0,005 m^2

Figure 1. shows the simplified flow-diagram of the equipment, on Figure 2. the static mixer can be seen inside the membrane tube. The experiments were carried out in cross-flow mode, the retentate was flowing back in the tank. The air flow was constant during the experiments, the transmembrane pressure varied between 0.5 – 3 bar.

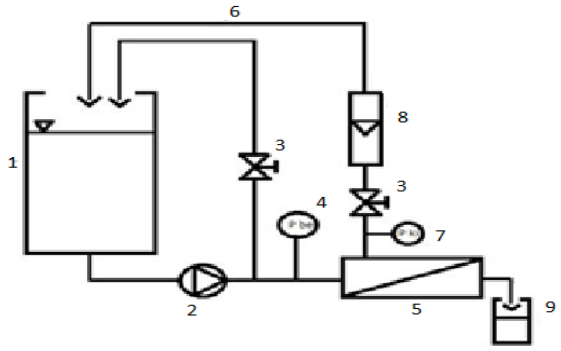

Figure 1 and 2: Flow-diagram of the equipment and the picture of the static mixer/membrane module (1. tank, 2. pump, 3. valves to controle pressures, 4. manometer, 5. membrane module, 6. permeate, 7. manometer,8. rotameter, 9. retentate)

3. RESULTS AND DISCUSSION

The results of the experiments for water flux measurement using aeration with static mixer (A+ SM), and using aeration without static mixer (A + NSM) can be seen on Fig. 3. and 4. In case of distilled water filtration we could reach higher flux valus because the higher shear stress values (Krstic et al., 2002).

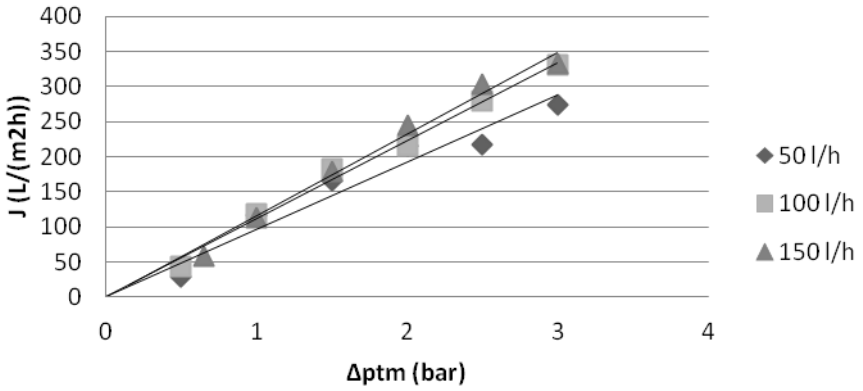

Figure 3: Distilled water flux result using static mixer, aeration (Δp_{TM}: 0.5 – 3 bar, Q_R: 50, 100, 150 L/h).

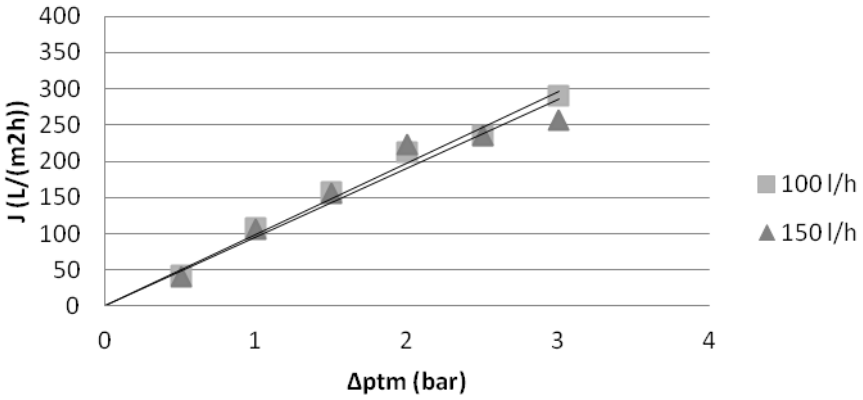

Figure 4: Distilled water flux result without static mixer, aeration (Δp_{TM}: 0.5 – 3 bar, Q_R: 100, 150 L/h)

On Fig. 5. and 6. the results of whey microfiltration can be found. The positive effect of static mixer can be seen on these diagrams. With static mixer using both 50 and 100 L/h recirculation flow rate the whey flux is increased. For long term fat removal experiments based on whey filtration results the maximum 2-2.5 bar transmembrane pressure (cricital pressure) is optimal because in case of higher pressure the flux will reach near constant value, it will not increase. Using static mixer helps increase the flux value, and probably the fouling phenomena will be reduced.

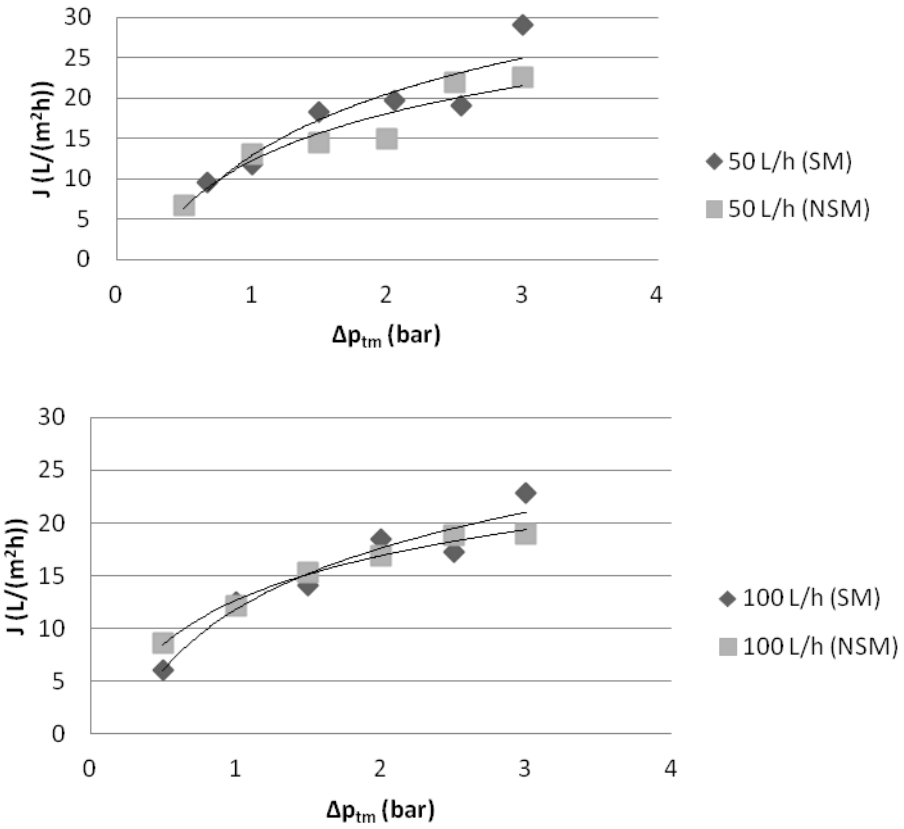

Figure 5 and 6: Whey flux result with (SM) and without static mixer (NSM) and aeration in case of 50 and 100 L/h recirc. flow rate (Δp_{TM}: 0.5 – 3 bar)

4. CONCLUSIONS

Comparing these results with the experiments' results done before (Kohány, 2012) lower fluxes values were reached. In the future we will have to measure the analytical composition of the permeate and retentates because we are expecting better retention values. Long term experiments will be carried out at 2 bar transmembrane pressure (critical pressure) and 100L/h recirculation flow rate. Pilot scale experiments will be done with static mixer, due to the results we were given, to intensificate whey microfiltration.

ACKNOWLEDGEMENTS: *The authors would like to express their acknowledgement to the Hungarian National Scientific Foundation TÉT 10-1-2011-0072, TÁMOP-4.2.1/B-09/1/KMR-2010-0005 and for MEMFIDA2 project (EUREKA_HU_08-1-2010-0010) for the support.*

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RECOVERY OF PINEAPPLE AROMA COMPOUNDS FROM MODEL SOLUTIONS BY VACUUM- AND SWEEPING-GAS PERVAPORATION

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SUMMARY
Our experiments were based on a model solution containing five of the main pineapple aroma components. Both sweeping-gas pervaporation and vacuum-pervaporation methods were carried out. Measurement were performed at different temperatures and feed flow rates. *The aims of this study were to examine applicability of the two pervaporation methods in reference to the pineapple aroma recovery, the effects of the operating parameters on the process and modeling the pervaporation process by* resistance-in-series model. The oprational conditions had favourable effect on the process. Higher enrichment could be reached with vacuum-pervaporation than the sweeping-gas method. The separation process is determined by the diffusion of *compounds in the membrane, thus the resistance in the boundary layer at liquid side is negligible. Based on performed experiments the pervaporation process can be applied in beverage industry for aroma recovery.*

1. INTRODUCTION

The aroma is one of the most important features of food, and is directly connected to the quality of the product and the consumer's acceptance. Some fruits contain hundreds of volatile flavours mostly in traces; however these chemical compounds could determine their basic characteristics (Aroujalian et al, 2007). The fruit juices are concentrated to reduce liquid volume, which results longer storage life of the product, moreover lower packaging and transport costs (Pereira et al, 2006). During juice concentration by evaporation process, losses of the aroma compounds can occur due to high temperature, thus deteriorating the quality of the concentrate (Börjesson et al, 1996). Rectification is a conventional method to preserve fruit flavors after evaporation of fruit juices. The major disadvantage of this traditional aroma recovery process is its high operating temperature, which causes very high energy comsumption. *In recent years, increasing attention is given to a membrane separation technique, the pervaporation.* There are a large number of studies examining the performance of the pervaporation process for the recovery of aroma compounds. *The pervaporation membranes are highly selective regarding the volatile aroma compounds, and their operation conditions are mild which gives the benefit of less space and energy need (Shujuan et al, 2005; Yang et al, 2006). In the future this process could be an important element in beverage and other industries too (Diban et al, 2008).*

2. MATERIALS AND METHODS

The experiments were carried out with model aqueous solutions of pineapple aroma compounds (Table 1). The measurements were performed at five different temperatures (30, 40, 50, 60 and 70°C) and three different feed flow rates (150, 300 and 600 L/h). The concentrations of aroma compounds were constant during all pervaporation experiments. The separation of aroma compound was operated by two organophylic membranes using both sweeping-gas pervaporation and vacuum-pervaporation methods. The PDMS ceramic tube membranes were collected in parallel in order to separate the model solutions with greater efficiency.Each experiment was run for two hours, while the permeate was condensed and collected in a cold trap by liquid nitrogen (-196°C). Concentration of permeate was determined by using a HP 5890 Series II. gas chromatograph equipment.

Table 1: Concentrations of pineapple aroma compounds in model solutions

Pineapple aroma components	ethanol	methyl-2-methyl butanoate	methyl-hexanoate	ethyl-acetate	i-amyl-alcohol
Concentration ppm	100	14	8,8	2	2

The characteristics of pervaporation can be described by factors like permeate flux and separation factor.

$$J_p = \frac{m_p}{A \cdot t}; \qquad \alpha = \frac{C'(1-C)}{C(1-C')}$$

Where J_p is the permeate flux [kg/(m^{2}h)], m_p is the weight of permeate [kg], A is the membrane surface [m^2] and t is the pervaporation time [h]. The α is the separation factor [-], C' and C are the concentrations of the aroma compound at permeate and feed side, respectively [m/m%].

The mathematical modelling of pervaporation can be desribed with the resistance in series model. The total resistance is the sum of the boundary layer resistances and the membrane resistance.

$$\frac{1}{Q_{OV,i}} = \frac{1}{Q_{L,i}} + \frac{1}{Q_{Mem,i}}$$

Where $Q_{OV,i}$ is the overall mass transfer coefficient of component „i" with driving force of partial vapour pressure [mol/(m^{2}Pas)], $Q_{L,i}$ is the mass transfer coefficient of component „i" with driving force of partial vapour pressure at liquid side [mol/(m^{2}Pas)], $Q_{Mem,i}$ is the mass transfer coefficient of component „i" with driving force of partial vapour pressure in membrane [mol/(m^{2}Pas)].

3. RESULTS AND DISCUSSION

Figure 1. „a" and „b" illustrate the effect of the operation temperature on the permeate fluxes of each aroma compounds at 600 L/h in case of both pervaporation methods.

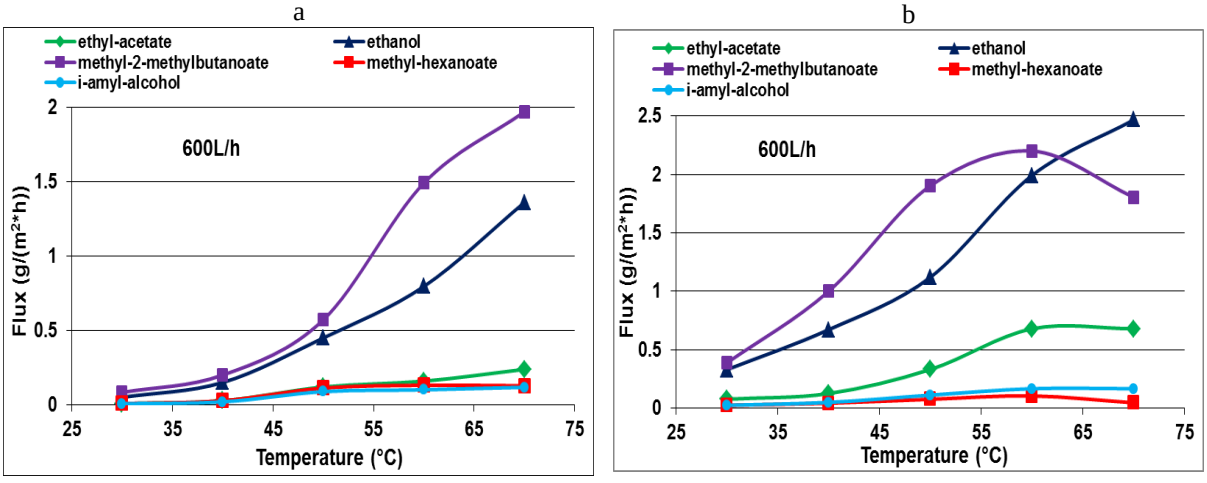

Figure 1: Effect of feed temperature on permeate flux at 600 L/h: sweeping-gas pervaporation (a); vacuum-pervaporation (b).

It can be seen that fluxes of the aroma compounds increase as the feed temperature was enhanced in case of pervaporation with carrier gas. Using vacuum pervaporation, instead of ethanol, the fluxes of aroma components show a maximum valeu at 60 °C. The higher permeate fluxes could be reached at 600 L/h with both separation methods.

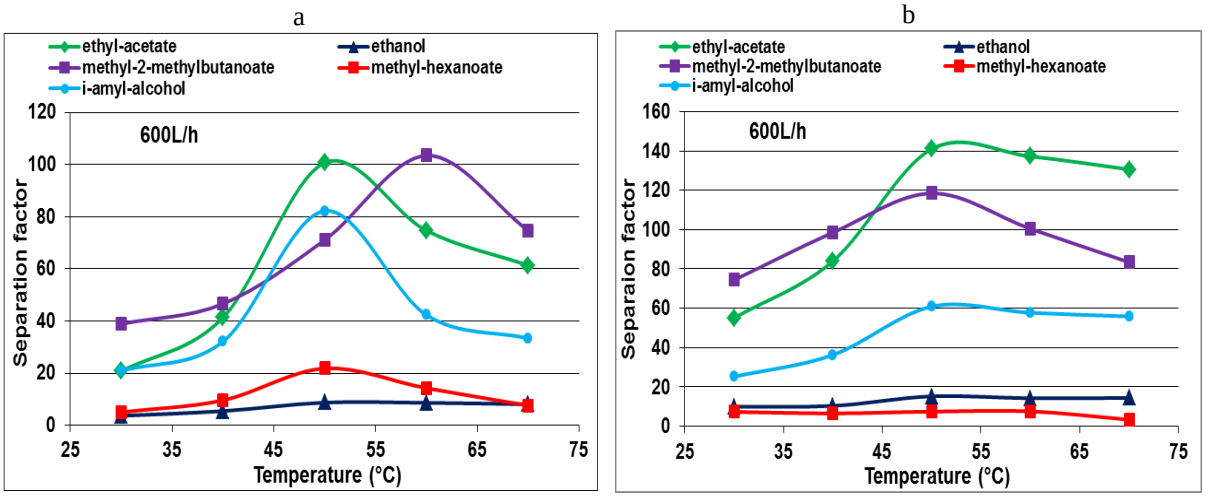

Figure 2: Effect of feed temperature on separation factor of the investigated pineapple aroma compounds at 600 L/h: sweeping-gas pervaporation (a); vacuum-pervaporation (b).

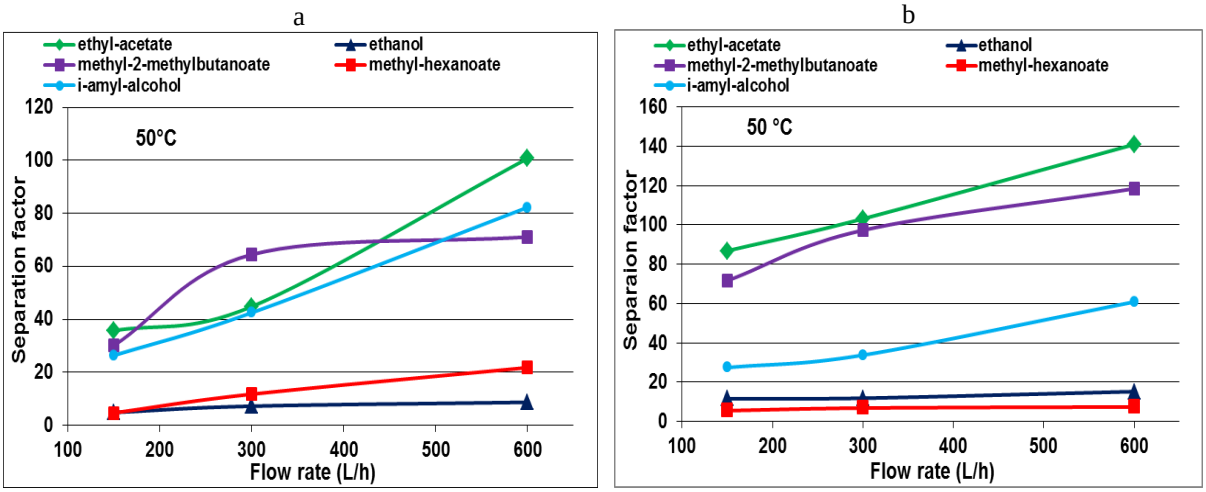

Figure 3: Effect of feed temperature on separation factor of the investigated pineapple aroma compounds at 50°C: sweeping-gas pervaporation (a); vacuum-pervaporation (b).

The separation factors are compared in figures 2 and 3 at the feed flow rate of 600 L/h and the feed temperature of 50°C in case of sweeping-gas pervaporation (a) and vacuum-pervaporation (b). Examining the values of separation factor selectivity increases with the feed flow rate and it shows a maximum at 50°C. Ethyl-acetate was separated with the highest efficiency. Most aroma components had higher separation factor using vacuum pervaporation method.

The calculated resistances for methyl-2-methylbutanoate in case of sweepig-gas pervaporation method are shown in figure 4. It can be noted that both overall and membrane resistances are much higher than resistances at liquid side. It can be established that the mass transfer is determined by the membrane, so the pervaporative resitance is influenced by only the structure of the membrane. This can also be observed in case of the other aroma compounds.

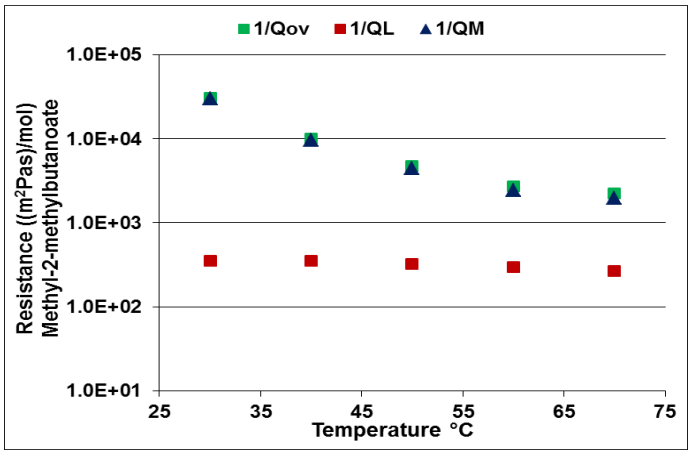

Figure 4: Resistance as a function of temperature related to methyl-2-methylbutanoate.

4. CONCLUSIONS

It can be concluded by the experimental data that both pervaporation methods are applicable for the recovery of the selected compounds from model solutions. Increasing temperature has favourable effect on the permeate flux, but selectivity shows maximum valeu at 50°C. Higher enrichment could be reached with vacuum pervaporation than the sweeping-gas method. The pervaporative resistance is effected on the resistance of membrane. This study generated the need for further examination to optimize some other operation parameters (feed concentration, pervaporation time) and investigation of aroma compounds recovery from real fruit juices.

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DETECTION OF COBWEB DISEASE *(DACTYLIUM DENDROIDES)* ON MUSHROOM *(AGARICUS BISPORUS)* CAPS WITH HYPERSPECTRAL IMAGING

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SUMMARY
Cobweb disease is one of the notable fungal infections, it caused serious losses in Europe. The aim of this study was to identify cobweb disease caused spoilage on the mushroom caps and find a method to discriminate between the infected and the undamaged samples.
Mushroom samples were divided into 2 groups. The first group was infected with cobweb disease and mushrooms were treated with three different fungicides. Mushroom caps were photographed and their hyperspectral images were acquired in the wavelength range of 900-1700 nm.
Infected and healthy areas were selected on the hyperspectral images, the main differences on these average spectra were found at the known water peaks (1200 and 1450 nm). The infected areas showed water loss, therefore the spatial distribution of the water content can be used for the detection of the spoilage. Support Vector Machine method was applied to discriminate between the infected and control groups and Monte Carlo cross validation was carried out.

1. INTRODUCTION

White button mushroom *(A. bisporus)* can be attacked by different pests and diseases: viral or bacterial diseases, microscopic fungi, insects, which cause great losses for the mushroom industry. Cobweb disease is a well-known fungal infection. The most important fungus species causing cobweb disease are *Cladobotryum dendroides* and *C. mycophylum*. (Győrfi, 2010). The name of this disease refers to its most typical symptom, when the cobweb-like mycelial growth appear on the surface of the fruiting body or the casing soil. The fruiting body becomes light brown and a shrinkage will be observable. Two types of spots appear on the cap: the first type is dark brown with indefinite contour, the second type is light brown with round contour. (Györfi, 2010)

Hyperspectral imaging (HSI) is a promising, rapid, non-destructive and remote sensing new technology. It can provide both spatial and spectral information from the object. This can be very useful if the distribution of a component or other feature is not homogeneous in the sample, therefore this technique became very popular in food quality measurements.

Previous studies (at Dublin Institute of Technology) investigated champignon with hyperspectral imaging to predict moisture content (Taghizadeh et al.,2009) and quality (in terms of moisture content, colour and texture) (Gowen et al.,2008a), to detect mechanical (Gowen et al.,2008b) or freeze damage (Gowen et al., 2009), to discriminate between casing soil, enzymatic browning and undamaged tissue (Taghizadeh et al., 2011) and to detect microbial spoilage, namely *Pseudomonas tolaasii* (brown blotch) (Gaston et al., 2011).
Since the cobweb disease causes spots on the surface of mushroom, that is why the spectral imaging method seems promising to detect infection, even in early stage.

2. MATERIALS AND METHODS

2.1. Mushroom samples

Mushrooms were cultivated and harvested under controlled conditions at Corvinus University of Budapest. Mushroom samples were divided into 4 groups: treated with 3

different fungicides (active substances: 1. natamycin, 2. prochloraz-Mn; 3. *Bacillus subtilis*,) and untreated. The groups were divided again and the first portion of the samples was infected with cobweb disease (before the harvest). The number of the samples in the 8 groups were varied, there were 61 infected and 39 not infected samples (untreated: 29, natamycin: 26 , *Bacillus subtilis*: 23 , prochloraz-Mn: 22 samples).

2.2. Hyperspectral imaging

After the harvest, mushroom caps were photographed using a digital camera. The hyperspectral images were recorded using a pushbroom HSI instrument (Headwall Photonics: Specim spectrograph, Xeneth InGaAs 14 bit sensor having 256*320 resolution) within the wavelength range of 900-1700 nm. The setup of optics finally resulted 5 nm spectral- and 0.475 mm spatial resolution.

The image processing system and the sensor were controlled by Argus hyperspectral software (Firtha, 2011). Before all measurement series, the signal of dark and bright standards were measured to calculate reflectance (spectral reflection factor) of samples.

2.3. Image segmentation and pre-processing of spectra

Infected and healthy areas (regions of interest) were selected manually on the hyperspectral images using CuBrowser Matlab algorithm (Firtha&Éder, 2012) and the average spectra of areas were saved.

To reduce the disturbing effects of non-homogeneous illumination (caused by the curvature of the mushroom caps), a simple normalization algorithm (the average of the intensities on the whole spectrum was substracted from the single intensity values) and a Savitzky-Golay smoothing was carried out on spectra.

2.4. Statistical evaluation

The model was built using RStudio version 0.97.336 software. The data of the infected and control samples were separated group by group, using Support Vector Machine method (e1071 package, developed at Vienna University of Technology).

Support Vector Machine (SVM) is a classifier method that performs classification tasks by constructing hyperplanes in a multidimensional space that separates cases of different class labels. To construct an optimal hyperplane, SVM employs an iterative training algorithm, which is used to minimize an error function.

Monte Carlo cross validation (MCCV) method was carried out with 20% of the samples as validation set (and 80% as training sample set) without optimisation. The iteration was repeated 10^3-times.

3. RESULTS

Figure 1. shows that there are real spectral differences between the control and the infected samples. The known water absorption peaks are noticeable (1200 and 1450 nm). Since at 1450 nm the infected spots show more reflection, so less absorption, we could guess, that these infected spots have less moisture content.

However at the other characteristic water absorption peak, we found opposite behaviour. Possibly because the change of this peak is much less, so the effect of normalization can be seen. Comparing two reflection factors at 1450 nm and around 1080 nm, seems to be characteristic to the infection.

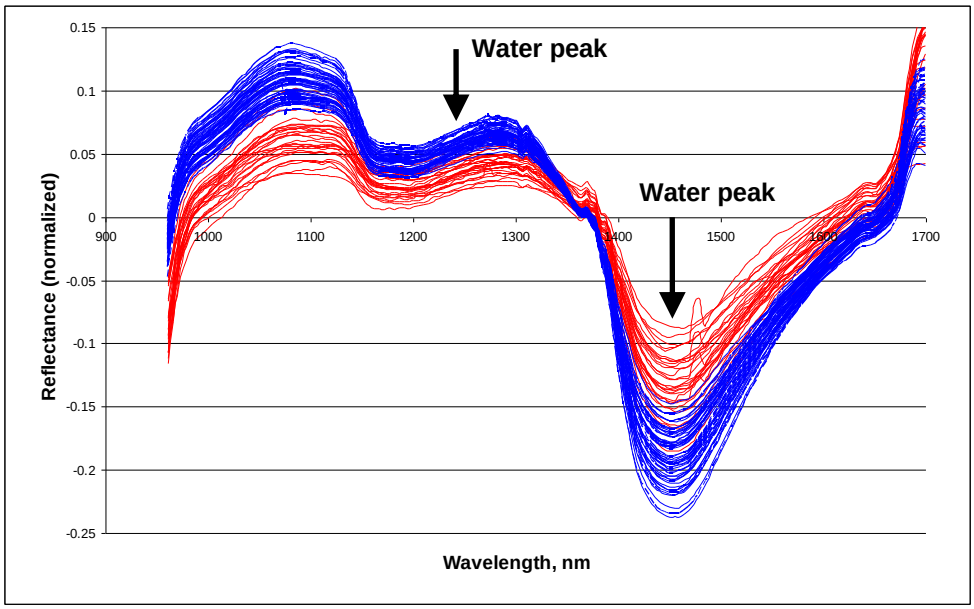


Figure 1: Measured spectra on the first day of an experiment, red: dactylium infected spot, blue: control

Figure 2. shows the changes of a natamycin treated sample at 1454 nm wavelength. On the normalized crossection of the hyperspectral images, the symptom of the disease appears as a light spot, mechanical damaged regions seem to be dark areas, so by this method the state of the tissue could be visualized and the cause of the damage is recognizable.

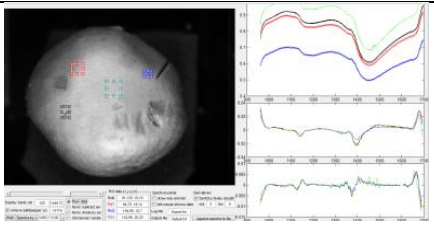
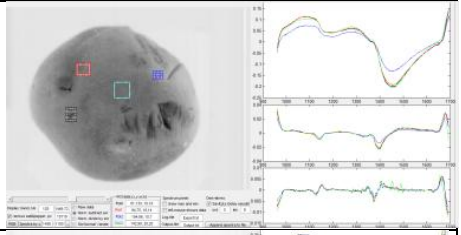
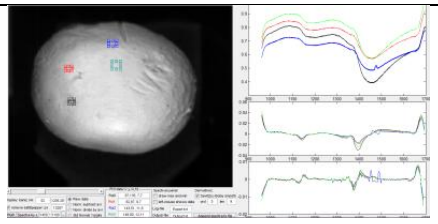
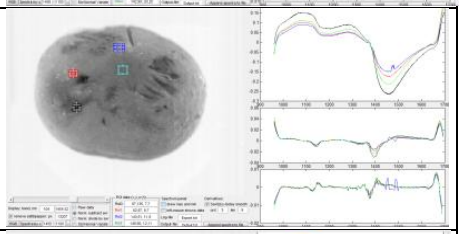
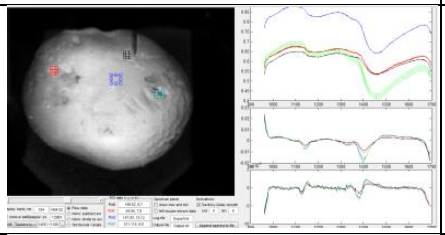
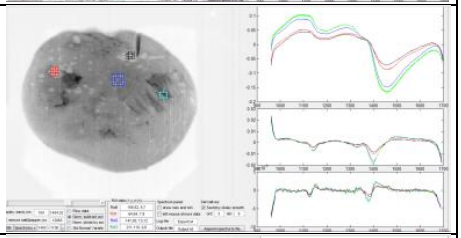
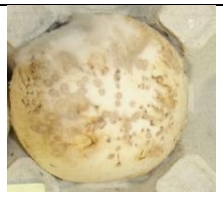
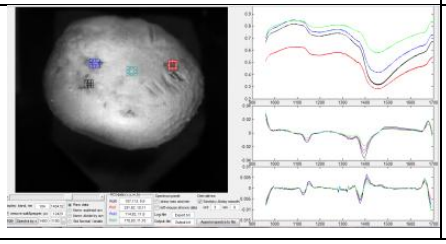
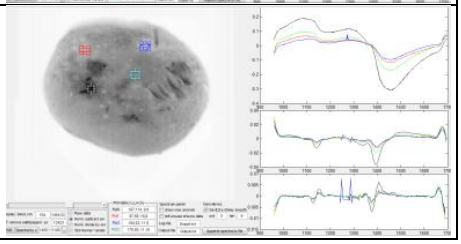
days	RGB images	1454 nm crossection	Normalized at 1454nm
1			
4			
7			
11			

Figure2: Images of a natamycin treated sample

The results of the classification with SVM method are shown in Table 1. The classification of the samples was successful. The correctly classified samples into not-treated, natamycin-treated, prochloraz-Mn -treated and *Bacillus subtilis* -treated groups were 100%, 80,78%, 89,71% and 93,16% respectively.

Table 1: Results of SVM classification

Not treated			Natamycin	
	true		true	
predicted	dactylium	control	dactylium	control
dactyilum	100.00%	0.00%	60.13%	5.60%
control	0.00%	100.00%	39.87%	94.40%
Correct classification	100%		Correct classification	80,78%
Prochloraz-Mn			*Bacillus subtilis*	
	true		true	
predicted	dactylium	control	dactylium	control
dactyilum	95.42%	16.96%	88.89%	4.24%
control	4.58%	83.04%	11.11%	95.76%
Correct classification	89,71%		Correct classification	93,16%

During the validation, this rate decreased slightly: in not-treated, natamycin-treated, prochloraz-Mn -treated and *Bacillus subtilis* -treated groups were around 98,5%, 78%, 88% and 91% respectively.

4. CONCLUSIONS AND PROPOSAL

The spectra of the infected samples showed differences from the control samples. On the normalized 1454 nm crossection of hypercube, the spoilage can be recognizable and its appearance is different from the mechanical damage.

The cobweb disease infected samples were successfully separated with SVM method, the ratio of the correctly classified samples was 80% in every group and over 75% in the validation process.

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ENHANCEMENTOF MILK MICROFILTRATION BY APPLICATION OF HELICALLY-SHAPED TURBULENCE PROMOTERS

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SUMMARY

Application of microfiltration process (MF) in milk concentration enables higher yields of cheese. Nevertheless, the process capacity can be low due to decrease of permeation flux due to fouling of membrane surface and/or pores. Fouling can be reduced by an increase of turbulence in a membrane channel. Turbulence in the membrane channel can be increased by inserting a motionless turbulence promoter (TP). Efficiency of promoter depends on its geometry. We have studied performance of three geometrical types of helically shaped TP-s: twisted tape (TT), tape with alternating helices (KM) and wire spring (WS). Experiments were performed on the 0.1 μm ceramic MF membrane using partially skimmed milk as the feed solution. Significant flux increase, three to eight times depending on the turbulence promoter geometry and cross-flow rate was achieved. Despite to the increased pressure drop along the membrane tube, energy savings are obtained in comparison to the operation without promoter.

1. INTRODUCTION

Concentration of milk proteins prior to the cheese production is important from the point of view of increase of cheese yield. Concentrate of milk proteins can be produced by application of membrane processes as are ultrafiltration or microfiltration (MF), in which concentration by factor of 20 is possible under the milder operating conditions compared to conventional thermal processes as is evaporation. Casein-enriched milk (retentate) prepared by MF has improved rennet coagulation properties and reduced loss of fat and fines in the whey (Skrzypek et al. 2010).

Efficiency of MF and UF processes is influenced by a decrease of permeate flux. Flux declines because of fouling of membrane surface and/or pores by proteins.Except of operating conditions such are cross-flow velocity and transmembrane pressure (TMP), formation of the membrane fouling depends of the characteristics of the feed solution and it is severe when the feed contains proteins. Due to a wide range of particle sizes in the milk, many of the particles deposited on membrane surface are difficult to lift. In removal and reduction of fouling caused by the mixtures of broad particle size hydrodynamic methods are effective. One of them is insertion of a turbulence promoter in membrane channel. Due to the presence of turbulence promoter in membrane tube, turbulence near the membrane wall increases, thereby reducing fouling of the membrane surface, but increasing a pressure loss along the membrane. Both, turbulence intensity and pressure loss depend on geometry and dimensions of a promoter. Different geometries of promoters have been tested in the membrane processes, especially in the MF of milk, such are KM Kenics^{TM} static mixer, semi-elliptical blade type mixer, helical screw-thread, and twisted tapes (Bellhouse et al. 2001; Krstić et al. 2002; Popović et al. 2013; Popović et al. 2011).

In this work the influence of three geometrical types of helically shaped TP-s: twisted tape (TT), tape with alternating helices (KM) and wire spring (WS) on the intensification of milk microfiltration was investigated. Those particular TP-s were chosen because they intensify turbulence in the similar way but cause the different losses in pressure along a membrane tube. Efficiency of TP application was evaluated in terms of the flux improvement (FI) and energy consumption (E).

2. MATERIALS AND METHODS

Experiments were performed using the laboratory scale microfiltration/ultrafiltration experimental unit, made of stainless steel (Fig. 1). Transmembrane pressure (TMP) across the module and flow were adjusted using the bypass valve and the main flow valve. Collected permeate was continuously weighted using the digital balance with the transfer of data to a PC. Experiments were conducted using a single channel ceramic membrane (GEA, Germany) details of which are given in table 1. As turbulence promoters twisted tape (TT), KM mixer and wire spring (WS) were tested of characteristics given in Fig. 1 and table 1. For all promoters dimension is expressed over an aspect ratio (O_{TP}) defined as the ratio of the pitch length (Le) to its diameter (D_{TP}) (table 1).

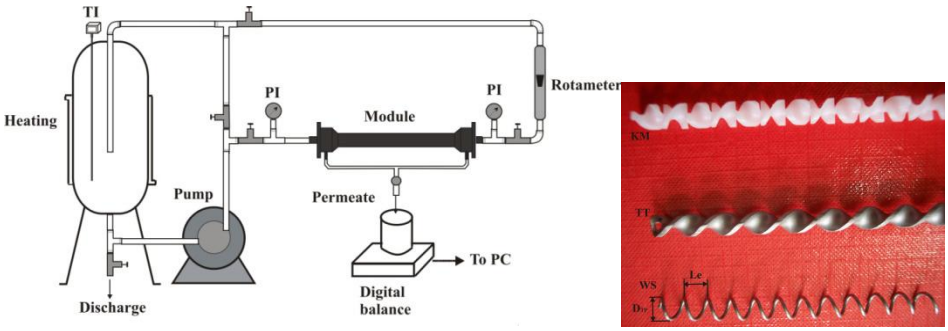

Figure 1:.Experimental unit and turbulence promoters.

Table 1: Dimensions of membrane and turbulence promoters

Membrane			Turbulence promoters		
			TT1.0	KM1.0	SW1.0
ID (mm)	6.8	D_{TP} (mm)	6.5	6.32	6.5
OD (mm)	10	L_{TP} (mm)	241	250	240
L (mm)	250	δ_{TP} (mm)	1.2	1.0	1.0
pore size (μm)	0.1	L_e (mm)	6.6	6.32	6.5
Active area (cm^2)	46.2	N_{TP}	36	40	36
Waterpermeab. L/(m^2hbar)	2600	O_{TP}	1.0	1.0	1.0

As feed solution pasteurized and homogenised, partially skimmed milk of the following composition (%, w/w): 3.1 proteins, 2.8 fat, 3.5 lactose and 0.7 ashes (Dukat Dairy Industry, Serbia) was used. All filtration experiments were performed with continuous recirculation of both permeate and retentate (volumetric concentration factor, VCF = 1) at temperature of 50±0.5 °C and TMP of 50kPa. Both, the MF unit and milk were preheated at 50 °C for 30 min before filtration. The filtration unit and membrane were cleaned according to the procedure: enzymatic cleaning (0.5% sold. P3-ultrasil 53), acid cleaning (1.0% sol. ascorbic acid), and alkali cleaning (1.0% sol. NaOH at 70 °C) with rinsing between each step. The membrane was considered as clean if more than 95% of the initial water flux was restored. Each experiment was repeated at least two times and standard deviation was not higher than ±5%.

Turbulence intensity is expressed over the Reynolds number

$$Re = \frac{vD\rho}{\mu} \qquad (1)$$

Where cross-flow velocity (v) was calculated dividing the applied cross-flow rate by a cross sectional area of an empty membrane.

The flux improvement(FI) represents a relative comparison of fluxes obtained with and without an application of promoter (Krstić et al. 2002):

$$FI = \frac{J_{TP} - J_{NTP}}{J_{NTP}} \cdot 100 \ (\%) \qquad (2)$$

A specific energy consumption (E) represents the hydraulic power dissipated (HDP) per unit volume of permeate:

$$E = \frac{HDP}{J_p A_{ac}} = \frac{Q\Delta P}{J_p A_{ac}} \qquad (3)$$

Energy savings potential was analysed calculating energy reduction in the reference to the operation without promoter:

$$ER = \frac{E_{NTP} - E_{TP}}{E_{NTP}} \cdot 100 \ (\%) \qquad (4)$$

3. RESULTS

3.1. Influence of turbulence promoter on fouling reduction

The curves of permeate flux (Fig. 2.) indicate type of fouling typical for the MF of milk irrespective of the mode of operation. Namely, a flux steeply decreases due to concentration polarization until after a short time it achieves a steady-state value. But, by using TP-s steady-state flux is increased several time depending on type of promoter and concentration polarisation is reduced. The most effective promoter is KM mixer increasing flux nine times in regard to the NTP operation under the same cross-flow rate.

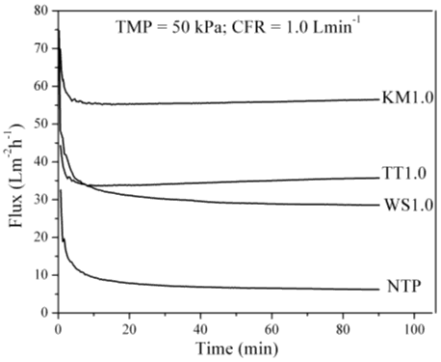

Figure 2: Time dependency of flux.

3.2. Influence of turbulence intensity

In Fig. 3 fluxes are presented as a function of Re number calculated using an initial velocity at the entrance of membrane. Real turbulence in membrane fitted with promoter differs due to differences in geometry of promoters. Nevertheless, for all modes of operation the steady-state flux increases with an increase of turbulence intensity. When compared at the same Re number the flux obtained by application of promoter is always higher than the flux obtained without promoter (NTP).

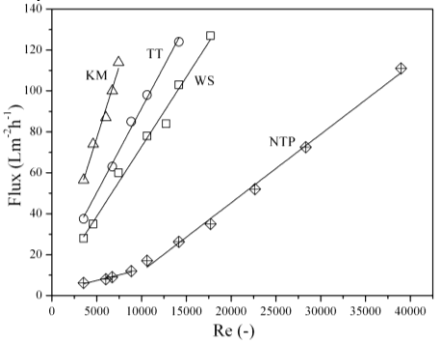

Figure 3: Turbulence intensity versus flux. TMP = 50 kPa.

An increase of turbulence intensity in the membrane fitted with promoter leads to fouling reduction. The main causes of turbulence intensification are the acceleration of fluid and an alternation of flow path. All three tested promoters alternate flow path to be helically shaped. WS is the least efficient because it does not force all the fluid to move towards the membrane wall while other two promoters do. KM mixer is the most efficient because it causes additional redistribution of fluid after each element while TT does not.

3.3. Flux improvement and energy consumption reduction

The highest flux improvement of 700-950% is achieved by application of the KM1.0 mixer (Fig. 4a). The other two promoters TT1.0 and WS1.0 delivered flux improvements of about 500 and 300%, respectively. Despite to the very high efficiency regarding the flux, the KM1.0 mixer causes much higher pressure loss than TT and WS under the same cross flow rate. A pressure loss in the membrane fitted with the promoter influences on the specific energy consumption as well.

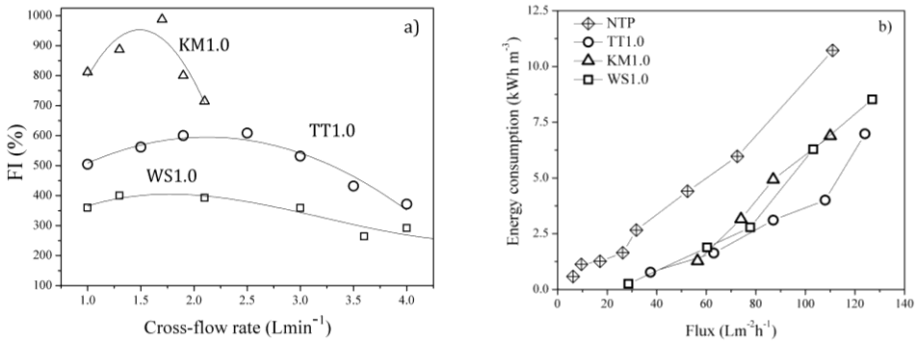

Fig.ure 4: Flux improvement a) and specific energy consumption b).

From an economic point of view the most important parameter is the specific energy consumption. It is obvious that the specific energy consumption gradually increases with an increase of the flux (fig. 4b). For the same permeate flux the specific energy consumption is always higher in the conventional MF without use of the promoter than that when the promoter is applied. For the lower flux values up to 60 $Lm^{-2}h^{-1}$ the specific energy consumption is almost the same for all three promoters. For the fluxes above 60 $Lm^{-2}h^{-1}$ the TT1.0 consumes less energy than the other two promoters and it the most efficient.

4. CONCLUSIONS

Application of TP increases the fluxes remarkably. The choice of mixer relies upon the compromise between the flux improvement and a pressure loss i.e. energy consumption. It is desirable to consider a promoter structure which will afford a long flow path so to avoid unfavourable deposition of fouling material, and which will enable scrubbing away the stagnant boundary layer on the tube wall. Also, this structure should not cause as high pressure loss so to hinder the flux improvement when the energy consumption is considered. The most efficient promoter is twisted tape enabling operation under the commercially acceptable flux of above 70 $Lm^{-2}h^{-1}$ with 50% reduced energy consumption compared to the NTP operation

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PROMISING VACUUM MEMBRANE DISTILLATION TREATMENT FOR OIL-IN-WATER EMULSION AS MODEL PRODUCED WATER PURIFICATION

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SUMMARY

In this present work a laboratory scale vacuum membrane distillation system has been applied to produce pure water from oil in water emulsion. Vacuum membrane distillation tests have been performed with various oil concentrations up to 250 ppm. Furthermore liquid entry pressure of pure water and oil emulsions in various concentration using two different methods (static and dynamic) has been also determined using commercial Durapore™ GVPH flat sheet membrane (Merck Millipore Inc., Billerica, USA) made of polyvinylidene fluoride.

1. INRODUCTION

Oily wastewater represents one of the greatest volumes of industrial liquid wastes. It is generated in amounts of millions of tons annually by worldwide industrial activities such as metal-finishing, machinery, aluminium, steel, textile, petroleum industry, and food processing as well. Its management presents considerable challenges and costs to operators. These oily wastewaters are mainly in the form of oil-in-water (O/W) emulsions that pose a great problem in facilities attempting to stay in compliance with discharge limits. A significant source of oily wastewater is the so-called produced water (PW). PW is water trapped in underground formations that is brought to the surface along with oil or gas. PW is considered as the largest volume waste stream in the exploration and production of oil and gas. Oily wastewater separation and PW purification is a potential candidate for MD. Low-grade heat from the PW actually can be utilized as the energy source to drive MD, because it contains energy that would otherwise be released to the environment without being used. This inherent thermal energy of the hot feed stream could be a boon to the energy required for the process.

This study presents experimental determination of liquid entry pressure (LEP) of a commercial flat-sheet membrane as a function of the dispersed oil content in model O/W emulsions as simplified produced water model solution. Two different methods have been applied for LEP determination using the same flat-sheet module configuration: the static method and the dynamic method. Furthermore, VMD process has been carried out to produce pure water from O/W emulsion and also from oilfield PW. The effect of the oil content determined as total organic compounds on the process performance and permeate quality has been also brought to light.

2. MATERIALS AND METHODS

2.1. Preparation and characterization of model oil-in-water emulsion

Model O/W emulsion in various concentrations has been prepared by using EmulsiFlex-C5 high pressure homogenizator at 50000 kPa (Avestin Inc., Canada, Ottawa). Distilled water and crude oil was used to prepare emulsions. Droplet size distribution and average droplet size of the feed emulsion was also determined using Mastersizer particle size analyser

(Malvern instrument Ltd, UK, Worcestershire). The average of the droplet size distribution in all prepared feed emulsion was 1.2-1.5 µm.

2.2. Description of static method for liquid entry pressure determination

Durapore^{TM} GVPH flat sheet membrane (Merck Millipore Inc., Billerica, USA) has been used for static LEP measurement based on Smolder et. al (Smolder, 1989). The membrane material is made of polyvinylidene fluoride. The nominal pore size of the membrane is 0.22 µm, and the active membrane area is 0.00444 m^2. Each test has used new membrane sheet. The measurements were carried out at room temperature. The upper part of the membrane module is filled up with feed solution to be in direct contact with the membrane for 5 hours. The outlet of the upper part is closed with a valve and a stagnant liquid layer formed at the top of the membrane. First, a low pressure is applied (0.2 bar) for 10 minutes using compressed air. Then, the pressure of the upper part is increased in every 5 minutes with 0.1 bar until the LEP was reached. At this moment, liquid starts to penetrate the pores and press out the air trapped there.

2.2. Vacuum membrane distillation measurements and description of dynamic method for liquid entry pressure determination

Experimental set-up of VMD system depicted in Fig. 1 has been used. Durapore^{TM} GVPH flat sheet membranes and module described in Sect. 2.1. was used. The feed liquid is recirculated with a flow velocity of 0.185 ms^{-1} and Reynolds number of 900 in upper channel of the flat-sheet module using a peristaltic pump (Watson-Marlow 501U, UK). Temperature of each test is fixed at 50°C using EKT Hei-Con contact thermometer (Heidolph Instruments GmbH, Schwabach, Germany) and downstream vacuum pressure is fixed at 70 mbar using diaphragm vacuum pump with controller (Model V-700/ V-850, Büchi Labortechnik AG. Flawil, Schwitzerland).

Following equation (1) is used to calculate the flux of VMD process.

$$J = \frac{1}{A}\frac{\Delta m}{\Delta t} \qquad (1)$$

Temperature and pressure sensors (Wika GmbH, Germany) as well as the digital balance are connected to Lab-manager (HiTech Zang GmbH, Germany) interface and the measured values are monitored by LabVision software (HiTech Zang GmbH, Germany). Each test has run up to 5 hours in batch mode.

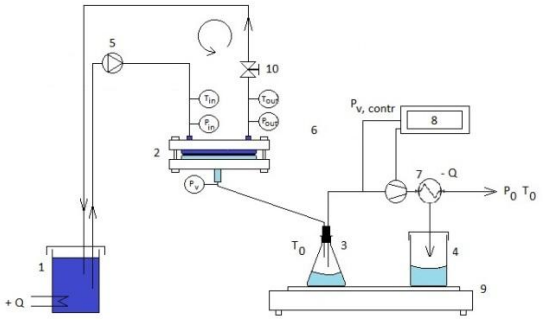

Figure 1: Schematics of vacuum membrane distillation using flat-sheet module with DuraporeTM GVPH flat sheet membrane (Merck Millipore Inc., Billerica, USA) using dynamic method (1. feed container, 2. flat-sheet module, 3. permeate condense collector before the vacuum pump, 4. permeate condense collector after the vacuum pump, 5. peristaltic pump, 6. vacuum pump, 7. cooler with tap water, 8. vacuum controller, 9. digital balance, 10. pressure regulator).

Same set-up depicted in Fig. 2 has been used for determining LEP values using dynamic method. The dynamic LEP measurements were carried out after every separate VMD tests using the same feed. Therefore a 5 hours long interaction of the feed on the membrane was carried out. Feed is circulating in the upper channel of the flat-sheet module. Flowing and operational parameters such as flow velocity, temperature and downstream pressure were the same as already has described. For increasing the pressure in the upper part of the module to reach LEP value, a pressure regulator is used at the outlet of the feed side channels. The feed side hydrostatic pressure is increased stepwise (0.2 bar) in every five minutes at the feed side. Once LEP is reached, the filtration performance of the membrane can be tested. The permeate flux of the wetted membrane is recorded at different pressures by using the balance placed under the permeate collector flasks and finally a hysteresis curve of the wetting phenomena can depict.

3. RESULTS AND DISCUSSIONS

3.1. Static method for liquid entry pressure measurements

Table 1 summarizes the results of the static method for LEP in function of emulsified oil content illustrated the physical appearance of the used membranes. It was found that up to 3200 ppm of oil content practically no effect was detected on the LEP value in function of the oil content. This value was detected around 2.35 bar. However a slight decreasing can observed at 100 and 1000 ppm, despite of, the results at 3200 bar (2.34 bar) suggest that this small fluctuation origins from another sources, probably from error of measurements. All the cases, the average of the droplet size in emulsions before and after the treatment was found in between1.2-1.5 μm. It means that the emulsion was stable during the tests and method had no effect on the quality of the emulsion in this 5 hours long period.

Table 1: Effect of oil content on the liquid entry pressure using static method at 25°C

Feed oil content, ppm	LEP, bar
0	2.365 (SD=0.015)
5	2.365 (SD=0.025)
100	2.325 (SD=0.005)
1000	2.29 (SD=0.010)
3200	2.34 (SD=0.010)

3.2. Effect of the oil content on the VMD flux

Effect of oil content on permeate flux of VMD was studied. Fig. 2 illustrates that up to 200 ppm of oil content; practically no effect of oil content on the VMD flux was observed. This value has been determined around 5 $kgm^{-2}h^{-1}$. In case of 250 ppm of emulsion, a spontaneous wetting was occurred, thus, 200 ppm was the upper limit of VMD using this configuration set-up operating parameters. Retention of the oil content was determined as membrane 91.3%, 94%, 97.6%, 97.29% and 97.27% at 30, 50, 100, 150, 200 ppm oil concentration, respectively.

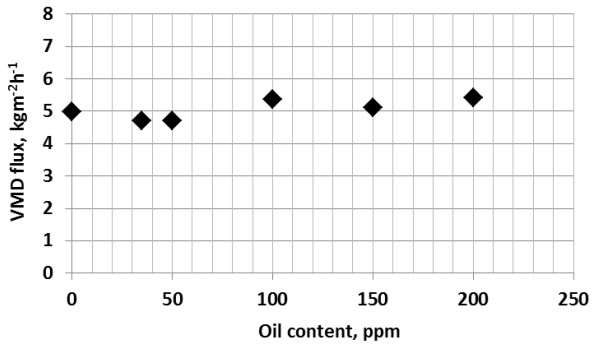

Figure 2. VMD flux in function of the oil content of the O/W emulsion (T_{Feed}= 50°C, v=0.185 ms^{-1} Re=900 p_v=70 mbar).

3.2. Dynamic method for liquid entry pressure measurements

Table 4 summarizes the results of LEP determination in function of oil content of O/W emulsion applying dynamic method. These tests were carried out directly after every separate VMD tests using the same emulsion. A negative effect of the oil content on the LEP value was determined. In details, at 0 and 35 ppm of oil content practically no effect was found: 2.0 bar of LEP was measured at both concentrations. This value of 2 bar transmembrane pressure difference was built up from 0.93 bar vacuum at the permeate side and 1.03 bar feed side. At 200 ppm, LEP value dropped down to 1.4 bar. Here the vacuum pressure at permeate side was also 0.93 bar thus the feed side pressure dropped down to 0.47 bar.

In case of composition of 250 ppm a spontaneous wetting occurred. During the circulation of feed emulsion approximately 0.3 bar pressure drop was measured along the feed side membrane length and no vacuum was applied. The membrane was already wetted under this condition using no vacuum pressure.

Table 1: Liquid entry pressure values in function of oil content of O/W emulsion at 50°C

Feed oil content, ppm	LEP, bar
0	2.0
35	2.0
200	1.4
250	Spontaneous wetting (0.3)

4. CONCLUSIONS

It was found that there is a difference in between the static and dynamic method for LEP determination. However, no effect was found on the LEP value in function of the oil concentration in case of static method up to 3200 ppm oil concentration, in case of the dynamic method a spontaneous wetting of the membrane pores was determined at 250 ppm oil concentration. Furthermore along in 0-250m ppm range, there was no effect on VMD flux identified in function of the oil concentration.

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APPLICATION OF ELECTRONIC TONGUE TO COMPARE THREE TYPES OF WHITE WINES FROM THREE DIFFERENT HUNGARIAN WINE REGIONS

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SUMMARY
The electronic tongue equipped with the specific sensor array is suitable to analyze wine samples. Linear discriminant analysis (LDA) was used to build models to separate white wine samples based on wine regions and grape variety as well. The results showed that every group was distinguished from each other with no misclassification error. Furthermore, the sequence of the wine sample groups was similar to the increasing 'acid content'. PLS was used to build models for the prediction of the evaluated chemical compositions of the wine samples based on the electronic tongue results. The closest correlation (R2=0.93) was found in case of 'acid content' and the prediction error (RMSEP) was 0.2. The pH of the wine samples was also predicted with close correlation (R2=0.89) and low prediction error (RMSEP=0.03) from the electronic tongue results.

1. INTRODUCTION

Nowadays quality assessment is an important topic in food quality control. Therefore, there is an increasing need for an instrument which can measure the quality or some quality parameters. The electronic tongue (ET) can be a useful tool in this field. The ET concept emerged first in the middle of the nineties (Kovács et al., 2009).The principle of the concept is to measure with cross-sensitive and partially selective sensors (Legin et al., 1999). The ET is a system that usually consists of an array of non-specific chemical sensors combined with appropriate data acquisition systems and chemometric tools. During sample assessment the ET sensor array produces an unresolved analytical signal, which correlates with the chemical composition of the sample.

Comprehensive information on the quality and composition of food products is becoming increasingly important for consumer choice. Geographical origin, agricultural practices and chemical composition, together with sensory qualities play a vital role in the purchase decision of consumers. These factors are even more important if we are talking about wines. From the point of view of wine quality, the chemical compositions play important roles. Chemical analysis of wine is a mature field of research and almost every type of modern advanced analytical technique has been applied to wine.

The result that we can obtain with ET is a chemical pattern characteristic for the definite sample. The similarities or even more the dissimilarities can be easily evaluated by the use of appropriate multivariate statistics (Brereton, 2009). Therefore, ET is a useful tool for comparative measurements *e.g.* comparison to a reference sample or validating the origin of a sample (Kirsanov et al., 2012).

The objective of our work was to demonstrate and prove the application potential of the electronic tongue for the evaluation of definite wine samples. Further aim was to compare the ET results to the results of chemical attributes.

2. MATERIALS AND METHODS

2.1. Materials

Nine white wine samples were analyzed during the experiment. The tested wine samples originated from three different Hungarian wine regions such as Balaton, Mátra, Villány. From each wine region there were three different wine samples. These wine samples were produced from different grape variety groups: Cserszegi fűszeres, Pinot Gris and Sauvignon Blanc.

2.2. Electronic tongue measurement

Alpha ASTREE II (Alpha M.O.S., Toulouse, France) potentiometric electronic tongue equipped with a specific sensor array was used to discriminate the white wine samples.

The ET consists of an autosampler, containing 16 slots for samples. During the assessment ISFET based potentiometric sensors were used. All measurements were performed with seven sensors (named ZZ, BA, BB, CA, GA and two HA according to the producer). The sensors were preconditioned before the tests performed by the electronic tongue. The preconditioning includes the actual conditioning and the calibration by the AlphaSoft software. For conditioning we used 0.01M HCl solution (recommended by the manufacturer.). The conditioning is performed according to AlphaSoft (Standard analysis). To achieve comparable results the electronic tongue has to be calibrated.

The calibration was performed with the mixed samples containing the 9 wines in the same percentage. Every sample was measured in nine replications. All measurements including both conditioning and calibration were performed at room temperature. The detailed description of the instrument was introduced in many previous publications (Kovács et al. 2010).

2.3. Statistical analysis

For the statistical data evaluation different statistical evaluation methods were used.

Principal component analysis (PCA) was used to detect patterns and to visualize the results of the electronic tongue tests. PCA is frequently applied as a tool to exploratory data analysis. Without losing useful information it is able to reduce multidimensional data sets to lower dimensions for analysis and to treat the outliers.

Linear discriminant analysis (LDA) as a supervised method applied to in order to evaluate the capability of discrimination of the current system maximizing the distances between classes by transformation of variables.

Partial least square (PLS) regression was used to determine relationship between the results of chemical data and electronic tongue tests. The validation was realized by leave-one-out (LOO) method. All analysis were carried out using the software R-studio 3.0 (Boston, USA) and Statistica 9.0 softwere (StatSoft, Inc., Tulsa, Oklahoma, USA).

3. RESULTS AND DISCUSSIONS

PCA was used as preliminary data evaluation for outlier detection (four outliers for each sample) and sensors selection. Based on discrimination ability sensitivy six sensors (ZZ, BA, BB, CA, GA and one of the two HA) were selected.

3.1. Classification by Linear Discriminant Analysis

Figure1 shows the results of discriminant analysis performed on ET measurement results to discriminate the nine different wine samples. The "Sauvignon Blanc of Balaton" shows the highest separation, from the other wine samples along Root1 having 81% of the variance. The group of the wine samples are located between "Pinot Gris of Mátra" having the lowest 'acid content' (4.5 g/L) and "Sauvignon Blanc of Balaton" having the highest 'acid content' (7.4 g/L). Consequently, Root 1 follows the order of increasing 'acid content'.

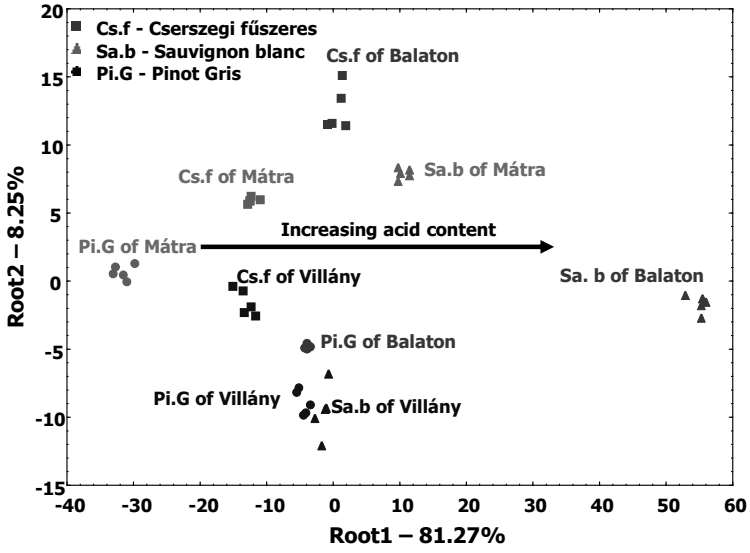

Figure1: LDA of the electronic tongue measurements performed with the wine samples

The corresponding LDA-classification matrix (data not shown), which includes the calibration and the validation (LOO), without any misclassification in the calibration model is observed during the wine regions and the wine types analysis. Figure 2a shows the LDA plot of the discrimination of the wine types. The wine samples made from different kind of grapes were clearly discriminated along Root1. Figure 2b shows the discrimination of the wine samples from different origin. The three wine regions (Balaton, Mátra, Villány) were definitely discriminated.

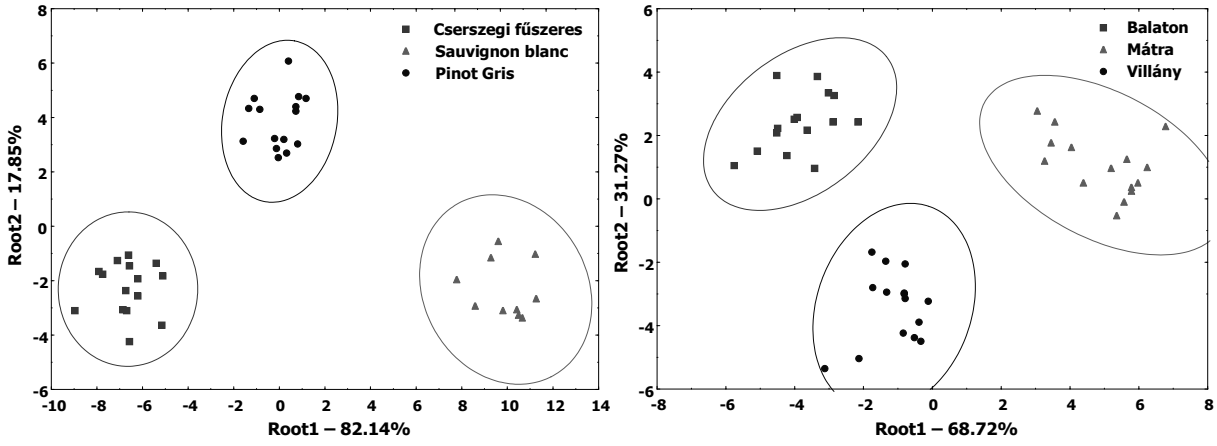

Figure 2: LDA plots of electronic tongue measurement to discriminate wine types (a) and wine region (b)

3.2. PLS regression model to predict some chemical attributes

PLS models were built to predict 'acid content', 'alcohol content', 'ash content' 'sugar free extract', 'density', 'volatile acid content' and 'pH' based on ET results. The determination coefficient and the prediction error of the PLS regression models are shown in Table 1. The closest correlation was found in case of 'acid content' (R^2=0.93) the prediction error was RMSEP=0.2. Relatively good correlation (R^2>0.85) was found in 'alcohol and ash content', 'sugar free extract' and 'pH'. The worst correlation coefficient was found in case of the prediction of 'volatile acid' content.

Table1. Parameters of ET performance for prediction of chemical attributes

	R^2	RMSEP
Acid content	0.93	0.2
Alcohol content	0.85	0.34
Ash content	0.88	0.13
Density	0.7	<0.01
pH	0.89	0.03
Sugar free extract	0.88	0.78
Volatile acid content	0.4	0.02

4. CONCLUSIONS

The electronic tongue equipped with the specific sensor array is suitable to analyze wine samples. Linear discriminant analysis performed on the electronic tongue results showed good classification of the wine samples in case of wine region and wine types as well. Furthermore, the order of the wine sample groups was similar to the increasing 'acid content'. Partial least square regression was used to build models for the prediction of the chemical attributes of the wine samples based on the electronic tongue results. The closest correlation was found in case of 'acid content' (R^2=0.93) the prediction error was RMSEP=0.2. The pH was also predicted with close correlation (R^2=0.89) and low prediction error (RMSEP=0.03) from the electronic tongue results.

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USING OF MEMBRANE DISTILLATION TO REMOVE CONTANIMANTS FROM WASTEWATER MODEL SOLUTION

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SUMMARY

The membrane distillation is the second step of a hybrid reverse osmosis (RO) – membrane distillation (MD) process where the feed in MD applications is the RO retentate.

As a first task in case of membrane distillation is to evaluate the effect of different configurations using distilled water then the concentration experiment have to be carried out to evaluate the rejection of the following compounds: arsenic, chromium, nickel, nitrate and ammonium ion. Based on the distilled water experiments the wastewater model solution concentration was carried out using counterflow configuration. As a result the ammonium ion rejection was over 80 % although it is a volatile compound. The rejections of heavy metals and nitrate ion were over 98 %, so the permeate of the wastewater model solution can be released into the sewage network. so the MD can be a proper tool where waste heat is available.

1. INTRODUCTION AND BACKGROUND

Membrane distillation is a possible solution for different metal ions (e.g. arsenic, chromium, nickel) removal from water or wastewater at high efficiency (Srisurichan et al., 2006). The separation of membrane distillation is based on the different volatility of the certain materials, and the driving force of the process is the vapor pressure difference. In principle only pure water vapor and volatile compounds permeate through the membrane, while the non-volatile compounds stay at the feed/retentate side (Criscuoli et al., 2013). The Direct Contact Membrane Distillation (DCMD) is one of the possible membrane distillation configurations, where hydrophobic, microporous membrane separate the heated feed side and the cold permeate side (Srisurichan et al., 2006). During the procedure the membrane surface may not be wetted by the liquid (water), at the interface the vapor molecules will diffuse through the membrane and condense on the cold permeate side. During the procedure also heat- and mass-transfer will be fulfilled through the membrane (Srisurichan et al., 2006; El-Bourawi et al., 2006).

The heat transfer is underlie MD process where the temperatures at the boundary layers of the membrane at both sides (feed and permeate) differ from the bulk solution (feed and permeate) and the heat transfer is went across the membrane by conduction. This phenomenon is called temperature polarization (Srisurichan et al., 2006; El-Bourawi et al., 2006; Gryta, 2007; Yarlagadda et al., 2011).which depends on membrane charasterics, feed velocity and concentration (Yarlagadda et al., 2011).

To investigate the driving force during the experiment, the heat transfer is determined by the logarithmic temperature difference (ΔT_{log}) (Eq 1).

$$\Delta T_{log} = \frac{\Delta T_H - \Delta T_L}{\ln \frac{\Delta T_H}{\Delta T_L}} \ (°C) \qquad (1)$$

where (in case of this configuration) ΔT_H (high temperature difference,℃) is difference between the temperature of feed and permeate inlet in direct flow, but in counter flow the ΔT_H is difference between the temperature of feed inlet and permeate outlet. However the ΔT_L (low temperature difference,℃) is difference between the temperature of feed and permeate outlet in direct flow, but in counter flow the ΔT_L is difference between the temperature of feed outlet and permeate inlet.

The mass transport is depending on volatility components at the feed side which are gone from the feed bulk to the feed boundary layer (at membrane surface) and transferred through

the membranes pores in gas phase, condensed to the bulk permeate solution (Srisurichan et al., 2006; El-Bourawi et al., 2006).

The mass transfer or permeate flux (J) across the membrane can be describable as:

$$J_i = \frac{\Delta m}{A \cdot \Delta t_i} \ (kg \cdot m^{-2} \cdot h^{-1}) \quad (2)$$

where A (m^2) is the membrane surface and m (kg) is the permeate mass collected over and the a period of time Δt (h) during the process.

The Reynolds number (Re) can be expressed in Eq. 3 in the inside and outside of the membrane, respectively. The density (ρ, $kg \cdot m^{-3}$) and dynamic viscosity (η, Pas) can be allocated by interpolation, where the d_e (m) is the equivalent diameter and the v ($m \cdot s^{-1}$) is the flow velocity.

$$Re = \frac{d_e \cdot v \cdot \rho}{\eta} \ (-) \quad (3)$$

Rejection was calculated according to following Eq. 4, where the c_R is the retentate concentration and the c_P is the permeate concentration.

$$R = \frac{c_R - c_P}{c_R} \cdot 100 \ (\%) \quad (4)$$

2. MATERIALS AND METHODS

2.1. Materials

Distilled water: To investigate the water flux of membranes and to find the best experimental set up experiments were carried out using distilled water as feed.

Wastewater model solution: In advanced the model solution was concentrated by reverse osmosis and its retentate was used during the processes by using the best set of experiment. The model solution was prepared using analytical reagent grade chemicals and distilled water. Arsenic sample solution was prepared by adding As [V] sodium salt heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$) and As [III] sodium arsenic ($NaAsO_2$) solution. Further components of the wastewater model solution: chromium was prepared from crystal chromium trioxide (CrO_3); nitrate was made by sodium nitrate salt ($NaNO_3$); nickel was prepared from nickel chloride solution ($NiCl_2 \cdot 6\ H_2O$) and the ammonium was made by ammonium chloride (NH^{4+}:NH_4Cl). The salt concentrations are summarized in Table 1. which can be found later in the text.

2.2. Membranes

During the experiments two different types of hydrophobic polypropylene membranes (Mycrodyn) were used: a hollow fiber and a capillary. In case of both membrane the pore size was 0,2 µm. Capillary module (MD 020 CP 2N): number of capillaries: 40, inner diameter: 1,8 mm, membrane surface: 0,1 m^2, membrane length: 0,5 m. Hollow fiber module (MD 020 FP 2N): number of capillaries: 250, inner diameter: 0,6 mm, membrane surface: 0,2 m^2, membrane length: 0,5 m.

2.3. Experimental design of membrane distillation

Four types of experimental configurations (a-d) were used with distilled water to investigate the water flux of the membranes and to find the best experimental set up; then wastewater model solution experiments were carried out to study the rejections. The feed (DW or model solution) was circulated inside capillaries/hollow fiber and the permeate was flowed outside capillaries/hollow fiber by peristaltic pumps (Multifix M 80). Temperature and mass were monitored by thermometers which were located before and after the membrane module (permeate and retentate side of the membrane); the mass difference/change was

observed using a scale (Sartorious PMA7500) which was connected to a computer. The temperature of the feed and the retentate were kept constant by using a thermostatic bath. The flow-rate was kept constant by the peristaltic pumps.

*a) Direct flow, MD 020 FP 2N***:** the feed side, before entering the module, was heated by hot water heat exchanger. Permeate side, before entering the module, was precooled with a tap water exchanger and went to the double glycol exchanger.

b) Direct flow, MD 020 FP 2N + cooling coil: the configuration was the same as the configuration a), but in the permeate side a cooling coil was added in which glycol was used as heat carrier.

*c) Direct flow, MD 020 CP 2N + cooling coil +precooled DW***:** the membrane module was changed and used precooled distillation water in permeate side.

d) Counter flow, MD 020 CP 2N + cooling coil + precooled DW: the membrane configuration was the same as configuration c) but the flow direction was altered.

3. RESULTS OF MD EXPERIMENTS

During the experiment the Reynolds number was always in the laminar region. By increasing the Reynolds number, the permeate flux is also increase, due to the reduction of the boundary layer resistance (The turbulent region is not suggested because could increase the risk of membrane wetting) (Criscuoli et al., 2013). The fluxes at different logarithmic temperatures are showen in Fig 1.

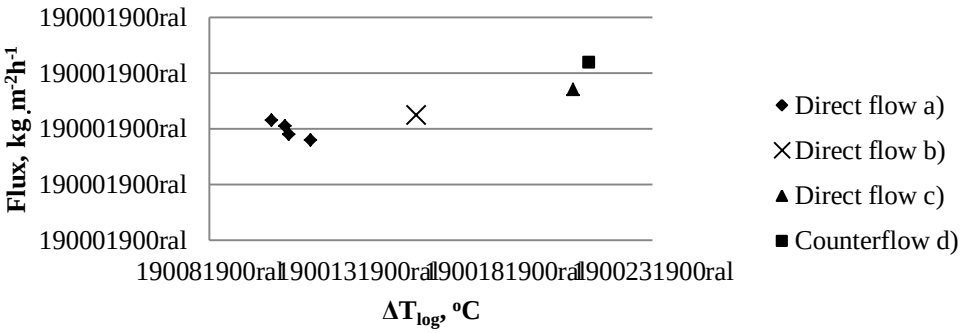

Figure 1: Flux values as a function of the logarithmic temperature difference at the different set ups in case of distilled water.

In the MD set up *a)* flux values were measured at the following flow rates: 20–20; 20–30, 30–20 and 30–30 ($L \cdot h^{-1}$) for the hot and the cold side, respectively. As a result the higher flow rate had a greater effect in the heated side. Because of this set up more components could diffuse through the membrane so it resulted as higher flux values.

At *b) version* ΔT_{log} was increased by the cooling coil, but in the flux was not caused notable changing.

After changing the membrane type (different geometric properties of the membrane) at *direct flow c) version* ΔT_{log} was further increased by the cooling coil and precooled distilled water. The system cooling efficiency was reduced by using the cooling coil and the precooled distilled water. The temperature of the permeate side was kept constant by the double glycol exchanger because the temperature rising of bulk by according to circulation was reduced.

At *counterflow d) mode* the ΔT_{log} was showed similar values than at the *c)* configuration, but the water fluxes were higher because of the flow direction changing.

As results of the counter flow mode was the most effective, while the feed was flowing outside of the membrane while the permeate inside the membrane. This mode kept the temperature difference between the feed and permeate constant keeping the mass transfer

alongside the membrane length stable. The wastewater model solution experiment is based on this set up *d)* mode.

Table 1: The experimental results of the wastewater model solution (MD 020 CP 2N, counter flow)

Name	Ammonium mg/L	Nitrate mg/L	Total Arsenic µg/L	Chromium µg/L	Nickel µg/L
Feed	56	347	3600	3020	1668
Retentate	71	530	5670	4440	1915
Permeate	12,64	4,08	110,11	<0,82	<0,82
Rejection %	82,19	99,23	98,06	>99,98	>99,96

The initial feed solution was compared to the retentate solution, it can be seen in Table 1, the concentrations of the contaminations were growing in the retentate. Concentration ratio ~2 was reached. The chromium and nickel compounds were under the limit of detection, the nitrate rejection was 99%, the arsenic rejection was 98% and in turn the ammonium ion rejection had the lowest value, 82%.

4. CONCLUSIONS

The principle of the membrane distillation the non-volatile compounds are retained in the retentate and the volatile species are went through the membrane micropores. During the experiment contaminations were found in the permeate (arsenic, nitrate, chromium), this phenomenon is explained by the partial wetting phenomenon. Membrane has larger pores will lead to a lower LEPw (liquid-entry-pressure of water) and it shows a hydrophilic property (Qu et al., 2009). Therefore the ions are transferred direct through the membrane to the permeate side. The rejection of metal ions and nitrate was 98 – 99%, where as the ammonium-ion rejection was under 85%. The ammonium is volatile compound and its boiling-point is lower than the water, so it is easily transferred through the membrane micropores, like water vapor. Therefore the membrane distillation is not recommended for the ammonium-ion concentration.

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A MEMBRANE FILTRATION-BASED CONCEPT FOR PRODUCING GALACTOOLIGOSACCHARIDES FROM WHEY

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SUMMARY

Galactooligosaccharides (GOS) are considered to be naturally occurring prebiotics. These low-calorie saccharides are claimed to selectively support the growth of beneficial microflora of our colon and thus improve our health. In addition to that, GOS also have excellent technological properties like low viscosity thermal and pH stability, high solubility and fine taste. The following multi-product process is proposed for the continuous production of GOS from whey: (i) defatting by microfiltration (MF) obtaining lipids as valuable raw materials for cosmetics, (ii) deproteinization of defatted whey by ultrafiltration (UF) forming whey protein concentrate and (iii) concentration and partial demineralization by nanofiltration (NF) Then, the concentrated lactose solution is used as feed for the enzyme membrane reactor (EMR) in which the enzymatic synthesis of GOS takes place. The so obtained product stream, that is rich in GOS, vitamins, bioactive molecules, minerals, and other valuable components, may serve as raw material for the formulation of low-calorie beverages.

1. INTRODUCTION

The demand for healthier and low-calorie foods is increasing worldwide. Our research deals with the development of a novel technology for manufacturing low-calorie, value-added beverages that have beneficial health effects. Our special attention is devoted to whey-derived lactose and its conversion to GOS. GOS are prebiotics because they can selectively stimulate the growth of beneficial bacteria in the colon. They also have excellent technological properties regarding to viscosity, stability, solubility and taste.

1.1. Whey

Whey represents a cheap source of lactose. Whey a by-product of cheese and casein production, is one of the biggest reservoirs of food. Whey comprises 50-90% of total volume of milk entering the process and consists about 50% of the nutrients in the original milk: soluble proteins, lactose, vitamins and minerals. Lactose forms the major solid part of the whey. Apart from the structured and functional proteins, whey also contains some non-protein nitrogen (NPN) like peptides, free amino acids, nucleotides, creatin, creatinine, urea, etc., which unlike true proteins, can even pass through the ultrafilter membranes (Bylund, 1995).

1.2. Membrane filtration

In the first phase of our research we investigate the filtration steps of whey.Membrane filtration can be defined as pressure driven membrane process for the separation and concentration of substances (Atra et al., 2005).

Cross-flow microfiltration (MF) is known as an efficient and energy saving process. In the dairy industry, cross-flow microfiltration is used for bacteria removal, fat removal, fractionation of milk proteins and separating of casein micelles and whey proteins. The major application of microfiltration is the pretreatment of whey to produce whey protein concentrate (WPC) during ultrafiltration (Razei et. al., 2010).

Ultrafiltration (UF) can be defined as pressure driven membrane process for the separation and concentration of substances having a molecular weight between 1 and 1000 kDa for ultrafiltration. Ultrafiltration can be used in the cheese industry to fractionate the proteins from whey and to make cheese from ultrafiltered milk (Razei et. al., 2010).

Nanofiltration (NF) is characterized by a membrane pore size between 0.5 and 2 nm and operating pressures between 5 and 40 bar. NF enables the partial demineralization of UF permeate and the concentration of lactose (Rasanen et. al., 2002).

1.3. Galactooligosaccharide (GOS) synthesis

Galactooligosaccharides (GOS) are considered to be physiologically favourable functional ingredients which promote the growth of beneficial bacteria int he colon. These carbohydrates are built up from glucose and galactose according to the formula Gal_n-Glc, where n = 2-20 (Kovács et. al., 2013).

GOS are produced by the action of β-galactosidase on lactose. GOS synthesis is a kinetically controlled reaction. The enzymatically catalyzed process depends upon several factors including the enzymes source the concentration of the substrate and reactions conditions such as pH and temperature (Kovács et. al., 2013).

2. MATERIALS AND METHODS

2.1. Whey

Dried whey was obtained from Kali – Szer Bt (Abrahamhegy, Hungary). In all experiments, the feed was prepared by dissolving 68 g of whey powder in 1 L of distilled water. The composition of this powder, as specified by the manufacturer, is listed in Table 1.

Table 1: Composition of whey

Protein, g/100g	Fat, g/100g	Water, g/100g	Solubility, g/10 cm^3	pH
13.04	0.5	2.03	0.1	6.57

2.2. Membranes

The properties and the conditions of membranes that were used for the filtration process are summarized in the Table 2. The fouled membranes were cleaned with 0.01 g/L NaOH and 0.01 g/L citric acid to regain the original fluxes.

Table 2: Membrane types and properties

Property	MF	UF	NF
Name	Durapore HVHP45	P010F	XN45
Manufacturer	Millipore, USA	Microdyn Nadir, Germany	Trisep, USA
Area (m^2)	0.125	0.06	0.046
Pore size (μm)	0.2, 0.45	0.1	0.1
Cut off	30	10	1
pH range	0 - 14	0 – 14	0 - 14
Pure water flux($Lm^{-2}hr^{-1}$)	>180	>150	>80
Max temperature (°C)	110	95	70

2.3. Enzymes

Reactions were carried out to convert the concentrated lactose obtained from the nanofiltration retentate to GOS. The enzymatically reaction were performed using the Pectinex Ultra SP-L and Lactozym 2600L enzymes at a concentration of 3 VV-1 %. Enzymes, Pectinex Ultra SP-L and Lactozym 2600L, both obtained from Novozymes A/S (Denmark).

The samples were analyzed for lactose, GOS, galactose, glucose using HPLC (Phenomenex, Rezex RNM carbohydrate column,; 45°C, 0.7 mL/min, Melz LCD312 RI detector).

2.4. Analyzes

Samples taken from the filtration train with Bently 150 Infrared Milk Analyzer (Bently Instruments, Inc. Chaska, MN, USA) for fat, proteins, lactose, minerals and NPN. The progress of filtration process were monitored by measuring the Brix, conductivity, TOC and pH.

2.5. Process design

Matlab (MathWorks Inc., USA) is a high-level language and interactive environment for numerical computation, visualization, and programming, were used to create models and applications. The process flowsheet was implemeted in the SuperPro-Designer (Intelligen Inc., USA).

3. RESULTS

The particular process operations and conditions were designed employing results of literature data and from our laboratory and semi-pilot scale investigations. The schematics of the overall process is shown Figure 1.

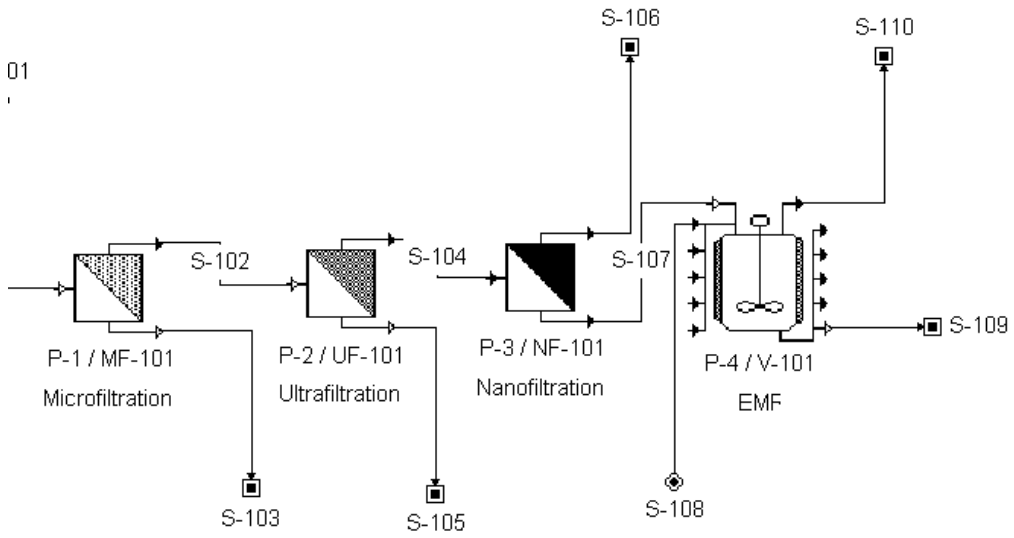

Figure 1: Scaled up design of the process

The final product of the filtration train is used as substrate for the enzymatic reaction with the two enzymes specified Sect. 2.3. It was aimed to achieve a high lactose concentration with in the NF retetntate.The whey first was microfiltrated with P-1/MF-101 to produce defatted whey, S-102. The permeate of microfiltration was the feed for ultrafiltration, P-2/UF-101. The ultrafiltrated permeate was used as feed for nanofiltration, P-2/NF-101. The concentrated lactose, which was produce with nanofiltration, was then the feed for the enzymatic membrane reactor. The reactor utilizes free enzymes for the production of GOS (S-109), Table 3.

Table 3: Characteristics of equipment and operations of outlet streams

Equipment	Code	Operation, stream	Composition g/L
Microfiltration	MF-101	P-1, S-102	Lactose 45.5; Protein 5.2; Fat 0; Minerals 5; NPN 1.7
Ultrafiltration	UF-101	P-2, S-104	Lactose 46.8; Protein 0; Fat 0; Minerals 5; NPN 1.6
Nanofiltration	NF-101	P-3, S-107	Lactose 125.9; Protein 0; Fat 0; Minerals 5.6; NPN 3.4
EMR	V-101	P-4, S-109	GOS 15%; Glucose 18%; Galactose 7%; Lactose 60%;

4. CONCLUSION

A following multi-product process is proposed for the continuous production of GOS from whey. The process includes (i) defatting by microfiltration (MF) obtaining lipids as valuable raw materials for cosmetics, (ii) deproteinization of defatted whey by ultrafiltration (UF) forming whey protein concentrate and (iii) concentration and partial demineralization by nanofiltration (NF) Then, the concentrated lactose solution is used as feed for the enzyme membrane reactor.

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PHYSICAL PROPERTIES OF MELONS DURING REFRIGERATED STORAGE

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SUMMARY

Trends of physical (rheological, acoustic, dielectric), physico-chemical (weight loss, pH, BRIX, water activity) and sensory (appearance, range test) properties of melons were examined during 17 days refrigerated (5°C) storage period. The fruits were bought from a local market-place in fully mature stage. The experiments were done on the whole (acoustic, weight loss) fruits and on the fresh-cut pulp (homogenized size cylinder). Samples were measured every 3-4 days. The physico-chemical (weight loss, pH, BRIX, water activity) changes show the ripening and spoiling of the fruits. The rheological trends show the softening of the pulp and the peel. The complex impedance changes at low frequency range (20 Hz – 10 MHz) could be related to the activity of pectin enzymes. The sensory tests give information about the acceptance by the consumers. One of the experimental targets was to establish relationship between these parameters and the non-destructive acoustic stiffness.

1. INTRODUCTION

Physicochemical and sensory parameters of different melon cultivars have been recently investigated in the literature (e.g. Cucumis melo L. var. reticulatus Naud. – Lester, G.E. and Turley R.M., 1990). Postharvest quality traits of fruits have been reported among netted muskmelon "fresh market" types (Evensen, K.B., 1983) and "western shipper" types (Davis and others, 1964, Lester and Turley 1990, Yamaguchi and others, 1977). The postharvest fruit quality of melons is variable and depends on the sugar concentration of the flesh because the melon fruit mesocarp tissue does not contain reserves of starch in fully maturity (Ryall and Lipton, 1979, Webster, 1975). Identification of an instrumental fruit harvest index for melon would assist the growers because these do not have visible maturity index, such as abscission zone development or exocarp colour change, immature fruits likely will be harvested along with mature fruits (Lester G. and Shellie K. C. 1992). Fruit maturity, as depend on starch and protopectin content, also exerted an effect upon texture (Paris and others, 2003), but the examination of these parameters means destructive, physico-chemical or chemical methods (Lester, 2006).

Impedance spectroscopy in a wide range of frequencies has been used earlier for monitoring the changes of vegetables during drying (Zsivanovits and Vozáry, 2011) and during long or short time controlled storage of apples and other fruits. The correlations between dielectric parameters in high frequency range (from 10 MHz to 1.8 GHz) and quality of melons were also analyzed by Wen-chuan and others, 2007, 2010). However, the prediction of soluble solid content by the dielectric properties was not as high as expected. They expect further studies for determining the practicality of sensing melon quality from the dielectric properties. Furthermore our team has reported some dependencies between dielectric parameters at low frequency range (20 Hz – 10 MHz) and short time storage parameters of fresh-cut apple and melon cubes packed in edible coating (Zhelyazkov S. and others, 2013, Zsivanovits and others, 2012). This study is a continuation of these earlier works.

2. MATERIALS AND METHODS

2.1. Experimental materials

The fully matured stage melon (Cucumis melo L. variety Magdemon) fruits were bought in the nearest local market-place from a primary producer, about 40 kg, 1-1.3 kg/piece (25 pieces) for that serial.

2.2. Sample storage and preparation

The whole melons were rinsed gently with tap water by hand and dried naturally. Then they were stored in refrigerator (controlled by datalogger for temperature and Rh; 5±1°C) for 17 days. The samples were examined on the first, forth, seventh, tenth, fourteenth and seventeenth days. The whole melons were weighed, and measured for mechanical parameters (3 outside diameters) and for acoustical stiffness. Each sampling days 4 pieces were selected randomly for further tests. After peeling, the pulp was cored and chopped for cylinders (d≈17 mm, h≈15 mm). The cylinders were used for rheological (penetration), dielectric, pH, BRIX, water-activity and sensory tests (used for the endpoint of the storage).

2.3. Experimental methods

The non-destructive stiffness (S) was measured by a computer supported audio signal analysis system and calculated by using:

$$S = f^2 \cdot m^{\frac{2}{3}} \quad (1)$$

Where *f* is the resonance frequency and *m* is the weight of melons in g. The values were expressed in stiffness units with dimension $10^4 Hz^2 g^{2/3}$ (Nourain and others, 2005).
The rupture point was analyzed by Stable Micro Systems penetrometer with a cylindrical head (d=25 mm). The maximum points of force-deformation curves were used for further analysis.

Impedance of the fresh-cut melon cylinders were investigated by GW INSTEK 8110G precision LCR meter in frequency range between 20 Hz –10 MHz with pin electrodes (gap 5 mm). Electric impedance and phase angle (θ) were used for the calculations in that experimental series. The real part (R_m), and imaginary part (X_m) of the measured impedance were calculated for monitoring the changing during the storage period:

If Z_m is a complex number, where:

$$Z_m = R_m + jX_m \ , \quad (2)$$

and R_m is the real part of Z_m and:

$$R_m = |Z_m| \cdot \cos\theta_m \ , \quad (3)$$

and X_m is the imaginary part of Z_m and:

$$X_m = |Z_m| \cdot |\sin\theta_m| \quad (4)$$

The maximum point of X_m-R_m diagrams, and their Rm and frequency values were used for statistical analysis (Vozáry E., and others, 2002).

The pH of the homogenized samples was determined by Microsyst MS2011 portable pH-meter, with temperature compensator. BRIX (abbe type refractometer), water-activity were examined by standard methods. The sensory ranking (with seven participants attending) of cylinders from different melons was used to expect the end point of the storage (ISO4121:2003(E)).

3. RESULTS

The near linear decreasing of dielectric parameters (fig. 1) such as the maximum of X_m and its R_m is expectable by spoiling of fruits (ruining of cell structure) like reported by other authors for fruits (Bico, 2009, Qi and others, 2011, Tapia and others, 2008). The relation is very similar for the rheological and hardness parameter as well. The exponential decreasing trends of the rheological and stiffness parameters (fig. 2) mean the softening of the melon texture which also has some enzymatic or respiration reason like it was reported in other works as well. The correlation matrix (table 1) shows high correlations between the physico-chemical parameters and the parameters of impedance, rheological and acoustic methods.

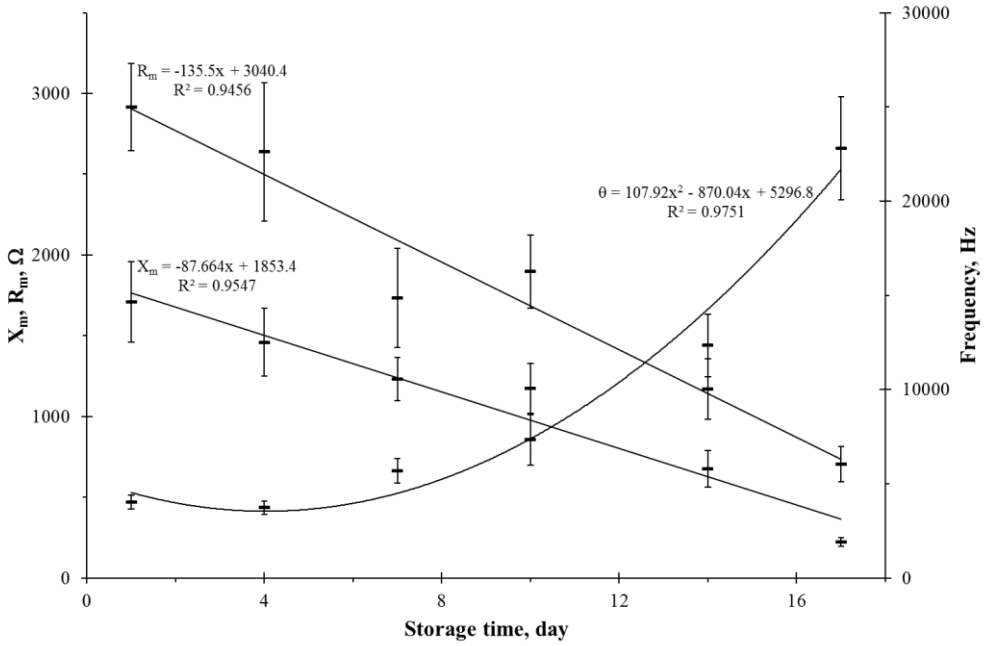

Figure 1: Impedance parameters

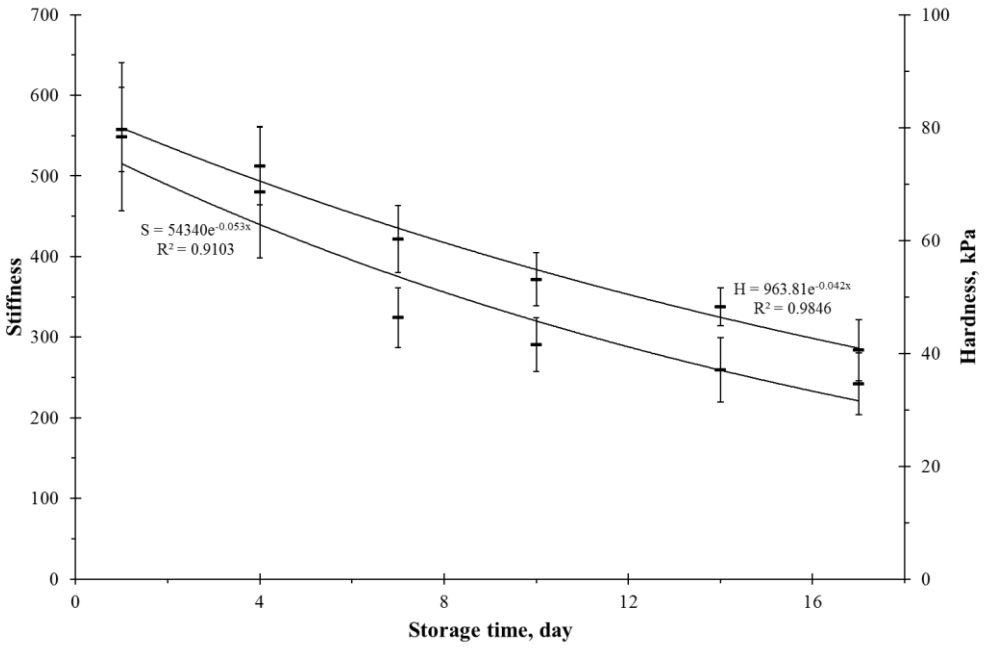

Figure 2: Acoustic stiffness and hardness

4. CONCLUSION, SUGGESTIONS

The similar exponential decreasing trends of destructing hardness (rupture point) and non-destructing stiffness parameters may mean a useful parameter for non-destructive quality prediction. The dielectrical parameters may also show the quality changes, but for stable correlations further experiments are necessary. The high correlations between the physical and nutritional parameters show these are useful for prediction the fruit quality.

Table 1: Correlation matrix on the parameters of impedance, rheological and acoustical methods

	Days	X_m	R_m	Frequency	Stiffness	Hardness	pH	Acidity
X_m	-0.9771	1						
R_m	-0.9724	0.9691	1					
Frequency	0.8980	-0.9570	-0.8840	1				
Stiffness	-0.9294	0.8564	0.9381	-0.7121	1			
Hardness	-0.9852	0.9406	0.9714	-0.8457	0.9738	1		
pH	-0.8046	0.8698	0.8094	-0.9557	0.6509	0.7145	1	
Acidity	0.8497	-0.9076	-0.7898	0.9597	-0.6537	-0.7340	-0.9595	1
Brix	0.8319	-0.7078	-0.6713	0.7300	-0.7950	-0.8508	-0.5105	0.5784

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BIOETHANOL DISTILLATION MODEL FOR POTENTIAL FARM-SCALE APPLICATION

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SUMMARY

Bioethanol is a renewable fuel which can be produced from several different biomass feedstocks and conversion technologies. Purification and distillation of bioethanol is an energy most consuming step in the production which is why numerous studies are directed toward improving and upgrading this procedure so that its use could be economically feasible in addition to the obvious environmental benefits of its application. In this paper, the possibility of new distillation model for concentrating of bioethanol from the fermentation broth is considered. Experimental results show an increase in ethanol concentration of 20% as well as reduction in energy consumption.

1. INTRODUCTION

Interest in use of biofuels, such is bioethanol, has grown strongly in recent years due to the limited oil reserves, concern about climate change from greenhouse gas emissions and the desire to promote rural economies (Balat and Balat, 2009). Biomass ethanol can be produced from a wide range of feedstock, which includes sugar-based (sugar beet or sugarcane juice and molasses), starch-based (corn and wheat) and cellulosic (bagasse and wood) resources (Grahovac et. al, 2012; Dodić et. al, 2009; Dodić et. al, 2010).

Several approaches have been proposed for the removal of ethanol from fermentation broths. They include vacuum fermentation, extractive fermentation and different membrane processes (Banat and Simandl, 1999). Although different separation techniques are available, distillation or distillation combined with other unit operations remains the main technology considered for the ethanol purification (Errico and Rong, 2012). The bioethanol separation process is significant in terms of its production cost. This can be solved either using heat recovering units or lowering the pressure at which the change of phase takes place. The first solution requires a somewhat complicated design to reuse the condensation heat and the second needs low pressure units that most of the times are expensive (Armenta-Deu, 2004).

The bioethanol fuel represents one important alternative to the fossil fuel for reduction of the carbon dioxide emission and therefore there is a need for a development of more energy efficient technology, equipment and distributive channels which can reduce the production cost and make the production of ethanol profitable for small farmers all over the World. Because of the small range of production, small farms have higher production costs than larger farms, mostly because of the high use of energy bought at market prices, which means they can't be competition on the market. The goal of this paper is to describe the basic concept of a model for bioethanol distillation suitable for small farms.

2. MATERIALS AND METHODS

2.1. Description of the model

The model, presented in Figure 1, contains two elements mutually connected through pipes into a closed system – the first is an evaporator (1) and the second is a condenser (2) simultaneously operating in a process of low pressure distillation and condensation and heat recovery. Each element consists of a cylindrical container and a centrifugal fan with an electrical motor (92 W, 230 V). The centrifugal fan produces low pressure in the container of both elements which decreases the boiling point of the liquid mixture or condensate. The evaporation and condensation processes are characterized by a liquid vapor interface through which mass and energy are transferred due to the existence of pressure, temperature and concentration gradients. The greater the difference between the evaporator and condenser temperatures the better the condensation process is.

In the lower part of the evaporator the liquid for distillation is heated whereas the upper part has a revolving lid connected to a centrifugal fan which channels the vapors into the condenser. During evaporation the accumulated steam is partially condensed on the evaporator lid while the rest of the steam (containing mostly ethanol vapors) is delivered through piping to the condenser where it partially condensates. In the evaporator, the liquid for distillation is connected through a peristaltic pump (3) (220 V, 40 W) to a modified sprayer whose opening is connected to the container and sprays to the revolving lid. Another sprayer which is part of the feed tank is connected to the lid of the condenser and contains liquid for distillation (4). Sprayers spray liquid mixture toward the revolving lid of each element.

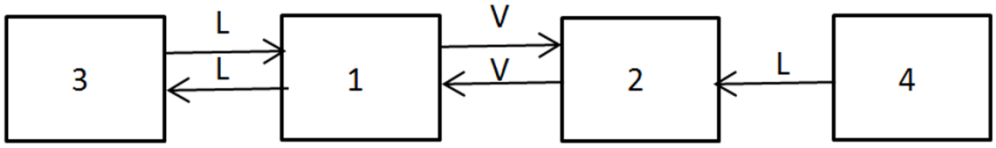

Figure 1: Block diagram of the new distillation model
1 – evaporator; 2 – condenser; 3 – peristaltic pump with sprayer; 4 – feed tank with sprayer;
V – vapor phase flow; L – liquid phase flow

2.2. Experimental conditions

Testing of the model was carried out with a 9.7% (v/v) ethanol mixture. Liquid for distillation in the evaporator (2000 mL) was heated until it reached a temperature of 60 °C after which it was transported to a sprayer with a peristaltic pump. In that moment both centrifugal fans were turned on as well as the sprayer of the condenser unit containing 2000 mL of the distillation liquid. Also, heating of the liquid for distillation was terminated and further process was conducted by recovering the heat through system.

During the experiment temperature of the liquid mixture and vapor phase below the cap in the evaporator and vapor phase in the condenser were measured. At the end of the experiment temperatures of obtained liquid mixtures in both evaporator and condenser were measured as well as the final concentration of ethanol in them (GC, 5890 series II, HP). All measurements were done in a triplicate and the average value was reported. Also overall consumption of energy was measured (Energy meter, PM 300, ETech). The experiment was finished after the content of the condenser sprayer was emptied.

3. RESULTS AND SUGGESTIONS

Results obtained in each repetition of the experiment are very close in value and are showing that the evaporation of ethanol from the distillation mixture occurred in applied experimental conditions and that vapors of ethanol left the evaporator through the centrifugal fan and partially condensed in container of the condenser unit. Results obtained through testing of the model were shown in Figure 2 and Table 1.

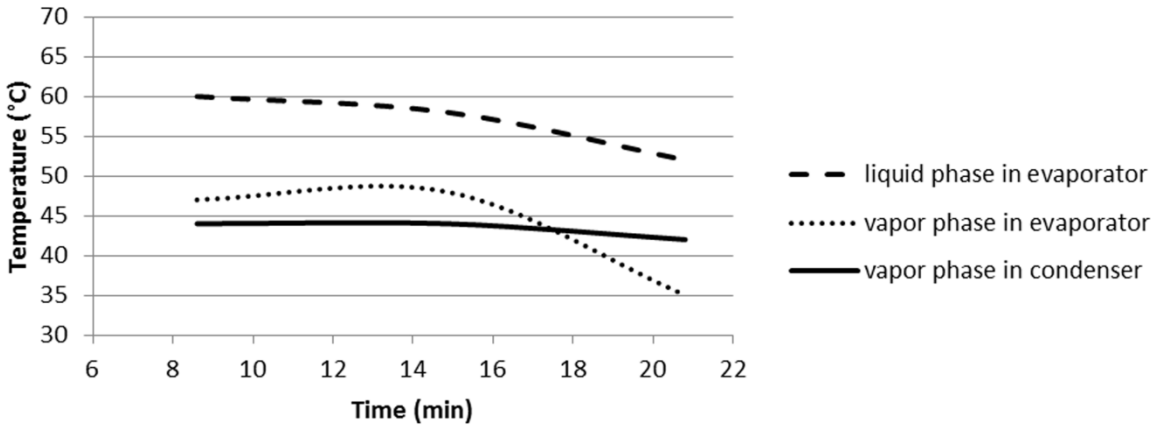

Figure 2: Temperature profile of liquid and vapor phase in evaporator and vapor phase in the condenser during experiment

Results obtained in each experiment are similar to one another and Figure 2 represents temperature profile of liquid and vapor phases in the evaporator as well as the vapor phase in the condenser obtained in one of the experiments. Temperature of the liquid phase in the evaporator decreased from initial 60 °C to the 52 °C which was expected because heating of the liquid for distillation was terminated after reaching 60 °C. The temperature of the vapor phase in the evaporator has a nearly constant value during the entire experiment. Temperatures of the vapor phase in the condenser are around 48 °C and are almost constant in the first half of the experiment after which it decreases to a temperature of 35 °C.

Table 1: Initial ethanol concentration of mixture (Ci), final ethanol concentration in evaporator (Ce), final ethanol concentration in condenser (Cc), energy consumption for heating and overall energy consumption

Run	Ci % (v/v)	Ce % (v/v)	Cc % (v/v)	Energy consumption for heating (kWh)	Overall energy consumption (kWh)
1	9.67	7.40	11.76	0.20	0.34
2	9.76	7.56	11.09	0.19	0.34

Results of ethanol concentration and overall and energy consumption used for heating are shown in the Table 1. Compared to the initial value of ethanol concentration in the mixture for distillation, which amounted to 9.7 % (v/v) in the evaporator a decrease in its concentration to about 7.5 % (v/v) took place, while the concentration of the mixture in the condenser increased to about 11.4 % (v/v). Although the increase in the concentration of ethanol obtained in the condenser has very low values because concentration increased by only about 2% (v/v) of ethanol, performed experiments showed that this new model has the potential to be used for distillation/concentration of the ethanol from liquid mixtures. Also, based on the possibility of recirculation of heat in the system of this model can be used for reducing the energy necessary for process of distillation. Furthermore, it is necessary to make

certain changes and enhancements of the model and to improve the efficiency of the distillation or concentration of ethanol in order to obtain products of satisfactory quality.

4. CONCLUSION AND SUGGESTIONS

With a described model for bioethanol distillation/concentration it is possible to increase the ethanol concentration in the medium for distillation while simultaneously recuperating the necessary thermal energy by recirculation in the system. However, although the concentration of ethanol increased comparing to the initial concentration in the mixture for distillation, further research and refinement of the model is required to increase its efficiency and the quality of the final bioethanol product, so it can be used as fuel (alternative energy source).

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POSSIBILITY OF XANTHAN PRODUCTION ON GLYCEROL CONTAINING MEDIA

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SUMMARY

Xanthan is the most important microbial polysaccharide industrially produced by Xanthomonas campestris. However, commercially available xanthan gum is relatively expensive due to glucose being used as the sole carbon source. Use of different wastewaters as less expensive substrate for biosynthesis reduces production costs and environmental problems. The aim of this study was to investigate the possibility of xanthan production on glycerol containing media. Process efficacy was determined based on xanthan yield and rheological characteristics of media. In applied experimental conditions, raw xanthan yields were in the range 9.90-15.88 g/L for media with initial glycerol concentration of 1-6 % w/v. Also, all cultivation medias showed pseudoplastic behavior. Obtained results suggest that glycerol has a great potential to be used as a carbon source in media for xanthan production.

1. INTRODUCTION

Xanthan is the most commercially produced microbial polysaccharide, obtained by *Xanthomonas campestris* cultivation. Widely used as a thickening or stabilizing agent in the food, pharmaceutical, and oil-recovery industries, this polymer is known for its unique rheological properties (pseudoplastic behavior), namely high viscosity at low shear, stability over a broad range of temperature and pH, and high resistance to shear degradation in aqueous solutions (Silva et al., 2009).

In the industrial production, price, rheological properties and yield of xanthan depends on the culture media used for the biosynthesis (Carignatto et al., 2011). Large scale xanthan production with high yield is usually performed on a glucose containing media. However, the increasing market price and demand suggest that glucose may no longer be economically feasible as a raw material. In order to reduce the costs of media for xanthan production use of different wastewaters as less expensive carbon sources are recommended. There are a number of research studies devoted to identifying a suitable substrate with low costs (Gilani et al., 2011).

Glycerol is the main by-product of biodiesel production. Generally, 10 to 20 % of the total volume of biodiesel produced is made up of glycerol. Growing biodiesel production will lead to large surpluses of glycerol. The significant amount of glycerol generated may become an huge problem, since it cannot be disposed of in the environment. Thus, there is an urgent need to find suitable applications of this waste (Quispe et al., 2013). One of the possible applications is its use as carbon and energy source for microbial growth in industrial microbiology. Glycerol may substitute traditional carbohydrates, such as glucose, sucrose and starch, in some biotechnological processes, e.g. production of ethanol, pigments, biosurfactants, citric, propionic and succinic acids (Da Silva et al., 2009). In this context, glycerol is an interesting potential substrate for xanthan production. Moreover, according to available literature data, use of this raw material as carbon source in media for xanthan biosynthesis has not been studied previously.

The aim of this study was to investigate the possibility of xanthan production by *Xanthomonas campestris* under aerobic conditions on media with different initial glycerol content. The rheological properties of the cultivation media, raw xanthan yield and degree of glycerol conversion into product were examined as indicators of success xanthan production.

2. MATERIALS AND METHODS

As a producing microorganism, for all experiments, the strain of *Xanthomonas campestris* ATCC 13951 was used. The strain was stored on YM agar slant at 4 ^{o}C and subcultured every four weeks.

The inoculum growth media was YM broth containing (g/L): glucose (15.0), yeast extract (3.0), malt extract (3.0) and peptone (5.0). In accordance with aim of this study, medias with initial glycerol content (g/L) of: 10, 20, 30, 40, 50 and 60 were used for xanthan production (media I-VI, respectively). These medias also contained (g/L): yeast extract (3.0), $(NH_4)_2SO_4$ (1.5), $MgSO_4x7H_2O$ (0.3) and K_2HPO_4 (3.0). The pH of the medias were adjusted to 7.0 and then sterilized by autoclaving at 121 °C and pressure of 2.1 bar for 20 min.

The xanthan production was carried out simultaneously in six Erlenmeyer flasks of 300 mL, each containing 100 mL of the media for biosynthesis with the appropriate composition (media I-VI). The inoculation was performed by adding 10 % (v/v) of inoculum prepared in aerobic conditions, on YM broth, at 28 °C in a laboratory shaker at 150 rpm for 48 h. The biosynthesis was carried out in batch mode under aerobic conditions for 7 days at the temperature of 30 °C and agitation rate of 150 rpm.

After biosynthesis, the media was centrifuged at 10000·g for 10 min in order to sediment the cells. Xanthan in the supernatant cooled at 15 ^{o}C was precipitated using 96 % (v/v) ethanol. Ethanol was added in small portions (1 drop per second) till the content of 60 % (v/v) with constant stirring. A saturated solution of KCl was added when half of the needed ethanol amount was poured into the supernatant in a quantity to reach a final content of 1 % (v/v). Obtained mixture was kept at 4 °C for 24 h and recentrifuged at 3500 rpm for 15 min. The precipitate was dried at 60 °C until constant weight to determine raw xanthan yield.

Rheological properties of the cultivation media samples were determined using rotational viscometer (REOTEST 2 VEB MLV Prüfgeräte-Verk, Mendingen, SitzFreitel) with double gap coaxial cylinder sensor system, spindle N. Volume of samples was approx. 15 mL. Based on deflection of measuring instrument, α (Skt), shear stress, τ (Pa), was calculated, under defined values of shear rates, from the following formula:

$$\tau = 0.1 \cdot z \cdot \alpha \quad (1)$$

where z is the constant with the value 3.08 (dyn/cm^2·Skt). According to Ostwald de Vaele equation, which describes viscosity of pseudoplastic fluids, and values of shear stress, rheological parameters were calculated.

3. RESULTS

The choice and concentration of carbon sources in media for xanthan biosynthesis are very important for the efficacy of production process (Leela and Sharma, 2000). In this study, six cultivation media with different initial glycerol content were examined for xanthan production.

The success of the performed biosynthesis was determined based on raw xanthan yield and degree of glycerol conversion into product. Obtained results are presented in Table 1. The highest product yield (15.88 g/L) was obtained from the media with maximal glycerol content (media VI). High yields were also achieved from the media IV (14.75 g/L) and media V (15.18 g/L). According to the literature data (Leela and Sharma, 2000), values of these results

are higher compared to the xanthan yield from media with 20 g/L glucose (14.74 g/L) which is the most frequently used carbon source in xanthan production. The smallest product yield came from media I (9.90 g/L), i.e. the cultivation media with the lowest glycerol content. On the other hand, in this media degree of glycerol conversion into xanthan was 98.96 %. This corresponds with results obtained from the literature that high degree of conversion (90 %), calculated on digestible sugars, is achieved when concentration of carbon source in media is less than 2 % w/v (Moraine and Rogovin, 1971). In the media II and media III, about 60 % and 45 % of carbon source, were converted into the product till the end of the process which is in accordance with the literature data (Rosalam and England, 2006). The lowest glycerol conversions into the desired product of about 30 % and 26 % were in the medias with highest concentrations of this nutrient.

Table 1: Raw xanthan yield and glycerol conversion into product

Media	Glycerol, S_0 [g/L]	Raw xanthan yield, P [g/L]	Conversion [%]*
I	10	9.90	98.96
II	20	12.02	60.10
III	30	13.65	45.49
IV	40	14.75	36.88
V	50	15.18	30.36
VI	60	15.88	26.47

* Conversion [%] = $P/S_0 \cdot 100$

Quality of xanthan produced in applied experimental conditions was evaluated based on the rheological behavior of cultivation media after biosynthesis. The rheological properties were determined from relationship between shear rate and shear stress shown in Figure 1. Flow curves represent pseudoplastic type of flow which is characteristic of xanthan solutions (García-Ochoa et al., 2000).

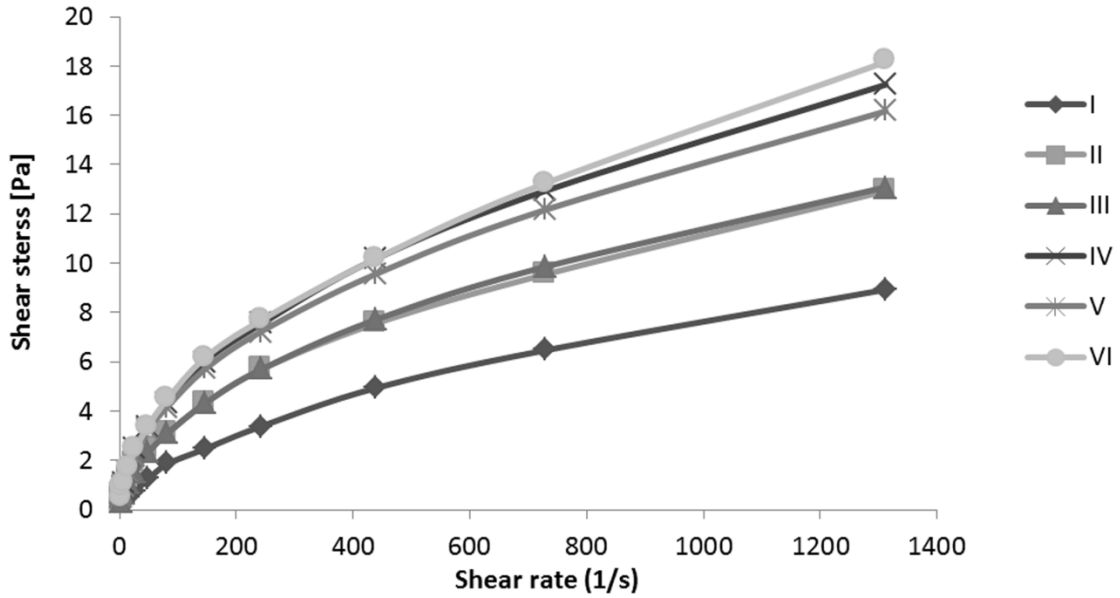

Figure 1: Effect of the shear rate on shear stress of glycerol containing media after biosynthesis

Pseudoplastic characteristics of glycerol containing media after biosynthesis are also confirmed by values of flow behavior index (n) and correlation coefficient (R^2) given in Table 2.

Table 2: Rheological parameters and correlation coefficient for glycerol containing media after biosynthesis

Media	K	n	R^2
I	0.0797	0.6757	0.9909
II	0.2114	0.5942	0.9823
III	0.1546	0.6470	0.9816
IV	0.2811	0.5960	0.9866
V	0.2702	0.5924	0.9889
VI	0.3221	0.5764	0.9893

Viscosity of xanthan solutions strongly depends on its structure and concentration (García-Ochoa et al., 2000). Given that the viscosity and consistency factor (K) are proportional, values of consistency factor (Table 2) indicate different quality and quantity of synthesized polymer. Values of flow behavior index, high values of consistency factor and results of product yield suggest that media IV, media V and media VI contained large quantity of xanthan with the good quality. These results and that obtained for media I, media II and media III indicate the possibility of xanthan production on glycerol containing media. Xanthan produced on media I has the lowest quality based on values of rheological parameters as well as the raw xanthan yield. However, there is no significant difference in the quality of the synthesized polymer on media II and media III, as well as media IV, media V and media VI. Obtained results are consistent with literature data according to which the preferred concentration of carbon source 20-40 g/L (García-Ochoa et al., 2000).

4. CONCLUSION

In this study, the possibility of using glycerol as carbon source in media for xanthan production was examined. Based on the pseudoplastic behavior of cultivation media and high values of raw xanthan yield (9.90-15.88 g/L) it can be concluded that the glycerol has a great potential to be used as a substrate for production of this valuable polymer. This research provided possible solution for use of crude glycerol which is abundant by-product of biodiesel production. The results obtained in this work could be the basis for optimization of xanthan production on glycerol containing media in order to reduce the negative impact on environment caused by disposal of this waste.

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OPTIMIZATION OF MEDIA FOR ANTIMICROBIAL COMPOUNDS PRODUCTION BY *BACILLUS SUBTILIS*

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SUMMARY

Bacillus subtilis is one of the most important producers of diverse antimicrobial compounds. These bacteria grow and produce antibiotics on different substrates. The increase of the antibiotics yield can be achieved by changing the conditions of cultivation and the composition of the culture media. In this study response surface methodology was used for optimization of glycerol, sodium nitrite and phosphate content in media for production of antibiotics effective against Staphylococcus aureus. For biosynthesis strain Bacillus subtilis ATCC 6633 was used. Developed model predicts that the maximum inhibition zone radius (38.08 mm) against Staphylococcus aureus and minimal amount of residual nutrients (glycerol 1.75 g/L, nitrogen 0.21 g/L, phosphorus 0.18 g/L) are achieved when the initial content of glycerol, sodium nitrite and phosphate are 49.99 g/L, 1.00 g/L and 5.00 g/L, respectively.

1. INTRODUCTION

In recent years, the increasing prevalence of numerous diseases has caused an urgent need to discover and develop new antimicrobial agents. Microorganisms represent inexhaustible source of bioactive compounds. Producing more than 70 different metabolites with antimicrobial action, including antibiotics, *Bacillus subtilis* is one of the most important producers. Some of its metabolites show antifungal and/or antibacterial activity against a number of pathogenic microorganisms (Kuta et al., 2009; Tabbene et al., 2009; Todorova and Kozhuharova, 2010).

Antibiotics production by microorganisms qualitatively and quantitatively depends on strain of the producing microorganism as well as on composition of the media and the biosynthesis conditions (Moita et al., 2005). However, culture media is of critical importance because variation of its composition can significantly effect on antibiotics yield. To achieve high product yields, it is necessary to properly define the composition of the media. Also, optimization of media is important from economic point of view because production cost extensively depends on the media composition as well as the unused nutrients. Optimization of media by the traditional "one-factor-at-a-time" technique requires a considerable amount of work and time. An alternative strategy is a statistical experimental design. Response surface methodology (RSM) is a group of statistical techniques for designing experiments, building models, evaluating the effects of factors and searching for the optimum conditions (Deepak et al., 2008).

The aim of this study is optimization of medium composition in terms of glycerol, sodium nitrite and phosphate content, by applying response surface methodology, for the production of compounds with antibacterial activity against *Staphylococcus aureus*. For antibiotics biosynthesis strain *Bacillus subtilis* ATCC 6633 was used.

2. MATERIALS AND METHODS

As the production microorganism strain *Bacillus subtilis* ATCC 6633 was used in these experiments. In accordance with the defined aim and experimental plan of the study, in medias for antibiotic production content of glycerol (20 g/L; 35 g/L; 50 g/L), sodium nitrite (1 g/L; 2 g/L; 3 g/L) and phosphate in the form of K_2HPO_4 (5 g/L; 10 g/L; 15 g/L) were varied. Medias also contained (g/L): yeast extract (0.5), $CaCO_3$ (17.0), $MgSO_4x7H_2O$ (0.5) and $MnSO_4x4H_2O$ (0.05). The pH value of the medias was adjusted to 7.0 ± 0.1 prior to autoclaving. The biosynthesis of antibiotics was carried out in an erlenmeyer flasks (300 mL) containing 100 ml of media. The inoculation was performed by adding 10 % (v/v) of inoculums prepared in aerobic conditions, on nutrient broth, at 28 °C in a laboratory shaker at 150 rpm for 48 h. Antibiotic production was carried out under aerobic conditions at the temperature of 28 ^oC and agitation rate of 150 rpm for 4 days.

At the end of process, samples of cultivation media were centrifuged at 10000 g for 15 minutes (Eppendorf Centrifuge 5804, Germany). In further work only liquid phase of cultivation media has been used. Standard methods were used for the determination of residual glycerol (Ph. Jug. IV 2, 1991), total nitrogen (Herlich, 1990) and phosphorus (Economic Review, 1990) content. After centrifugation, supernatants were concentrated by evaporation on rotary vacuum evaporators (Ika-werke, Staufen) to one tenth of the initial mass. The antibacterial activity of obtained samples against *Staphylococcus aureus* was tested, *in vitro*, by diffusion - disc method (Bauer, 1966).

The selection of an experimental plan is a key step in the application of the RSM. In this study experiments were carried out in accordance with Box-Behnken design with three factors on three levels and three repetitions in the central point. The factor variables are and their values are: X_1 glycerol content (20-50 g/L), X_2 sodium nitrite content (1-3 g/L) and X_3 phosphates content (5-15 g/L). For the description of the responses Y (residual glycerol (g/L), residual nitrogen (g/L), residual phosphorus (g/L) and inhibition zone radius (mm)), a second degree polynomial model was fitted to the data:

$$Y = b_o + \sum b_i X_i + \sum b_{ii}^2 X_{ii}^2 + \sum b_{ij} X_i X_j \quad (1)$$

where b_0 represents intercept, b_i represents the linear, b_{ii} quadratic and b_{ij} interaction effect of the factors.

Statistical analyses of experimental results were performed using Statistica software v. 12.0. For determination of optimal values of examined factors, method of desired function was applied (Design-Expert 8.1).

3. RESULTS

In accordance with aim of this study and applied experimental plan, medias for antibiotics production were formulated by varying content of glycerol, sodium nitrite and phosphate. For responses obtained after these experiments, a polynomial model of the second degree was established to evaluate and quantify the influence of the variables. The adequacy of the model was evaluated by determination coefficient (R^2) and model *p*-value. The results of the statistical analyses are presented in Table 1.

Relatively high values of R^2, obtained for all responses, indicate good fit of the experimental data to Equations 1. A *p*-value is used to assess the statistical significance for each of the regression equations' coefficients. Regression coefficients, with a significance level of 95 %, are significant if the value of their *p*-value is less than 0.05. These coefficients are bolded in Table 1.

Table 1: Regression equation coefficients, their significance and determination coefficient for selected responses

Effects	Residual glycerol		Residual nitrogen		Residual phosphorus		Inhibition zone radius	
	Coefficient	*p*-value	Coefficient	*p*-value	Coefficient	*p*-value	Coefficient	*p*-value
intercept								
b_0	**-11.1413**	**0.0429**	-0.0019	0.9892	-0.1633	0.6603	**-36.2685**	**0.0497**
linear								
b_1	**0.5906**	**0.0080**	-0.0016	0.7348	**0.0455**	**0.0117**	**3.4963**	**0.0007**
b_2	1.7108	0.4137	0.1581	0.0586	-0.2078	0.2573	-0.5417	0.9372
b_3	0.3225	0.4392	0.0240	0.1233	-0.0761	0.0662	-2.1250	0.1654
quadratic								
b_{11}	**-0.0080**	**0.0052**	0.0001	0.5806	**-0.0005**	**0.0132**	**-0.0352**	**0.0017**
b_{22}	-0.1321	0.7439	0.0058	0.6682	0.0152	0.6583	-0.6667	0.6310
b_{33}	-0.0041	0.8002	-0.0007	0.2089	**0.0036**	**0.0385**	0.0533	0.3536
interaction								
b_{12}	0.0205	0.4410	-0.0007	0.4361	-0.0015	0.5023	-0.0833	0.3643
b_{13}	0.0006	0.9022.	0.0000	0.9999	-0.0006	0.1848	-0.0033	0.8497
b_{23}	-0.1785	0.0595	-0.0038	0.1815	**0.0281**	**0.0062**	0.6000	0.0621
R^2	0.912		0.974		0.956		0.979	

The most significant response, for antibiotics yield, is inhibition zone radius against *Staphylococcus aureus* because it is a direct indicator of the amount of synthesized antimicrobial compounds. For this response, coefficient of determination was found to be 0.979, which indicates that only 2.1 % of the variations could not be explained by the model. As for significance of the polynomial coefficients, their *p*-values suggest that the most important linear factor is initial content of glycerol and the same conclusion can be applied to quadratic effects. Considering that carbon sources are involved in primary and secondary metabolism of microorganisms, these results are expected. Therefore, in applied experimental conditions, glycerol, as a carbon source, is the most important nutrient in media for the production of antibiotics effective against selected test microorganism.

The final goal of response surface methodology is the process optimization. Thus, the developed models can be used for simulation and optimization. To optimize the process with two or more output responses, it is helpful to use the concept of desirability function (D) (Grahovac et al., 2012).

In this study, four responses were used for optimization of antibiotics production i.e. maximization of inhibition zone radius against *Staphylococcus aureus* while residual content of glycerol, total nitrogen and phosphorus was minimized. Effects of glycerol and sodium nitrite content on the value of desirability function at the optimal phosphate content (5 g/L) are presented in Figure 1. As it can be seen from Figure 1, the highest values of desirability function are obtained in the region of high initial glycerol content and low initial sodium nitrite content. The optimal values of initial content of glycerol, sodium nitrite and phosphate are 49.99 g/L, 1.00 g/L and 5.00 g/L, respectively, for the highest value of desirability function (0.958). The optimal responses values for selected initial nutrients content are 38.08 mm, 1.75 g/L, 0.21 g/L and 0.18 g/L for inhibition zone radius against test microorganism and residual content of glycerol, nitrogen and phosphorus, respectively. Minimal optimal content of sodium nitrite and phosphate confirm that the nitrogen and phosphorus are commonly the

major growth-limiting nutrient and play an important role in the onset and intensity of secondary metabolism in which antimicrobial compounds are synthesized (Sanchez and Demain, 2002).

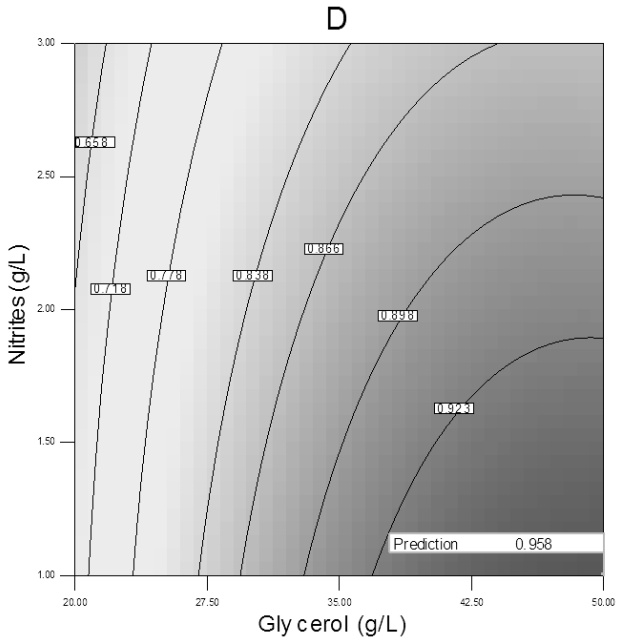

Figure 1: The overall desirability function of the antibiotics production process

4. CONCLUSION

This research has confirmed that *Bacillus subtilis* ATCC 6633 has great potential for the production of antimicrobial compounds effective against *Staphylococcus aureus*. Response surface methodology proved to be efficient tool for optimizing process conditions, relatively simple and time and material saving. The regression equations obtained in this study can be used to find optimum conditions for antimicrobial compounds production in industrial scale from economic point of view.

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FRUIT JUICE FERMENTATION WITH BIFIDOBACTERIA

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SUMMARY

Consumers are becoming more interested in healthy nutrition. To meet consumer requirements the possibility of the fruit juice fermentation by bifidobacteria was investigated. Sour cherry and orange juice was fermented with five Bifidobacterium strains (from human origin and starter culture). The tested strains have grown better in orange juice. The B. longum Bb-46 strain demonstrated the best growth activities. Due to metabolisms of the strains the concentration of formic acid, succinic acid, lactic acid and acetic acid increased during fermentation. Concentrations of oxalic and citric acid did not change. In course of the fermentation the antioxidant capacities slightly decreased, except when the orange juice was fermented with B. lactis Bb-12 and B. longum A4.8. The obtained results may contribute to the design a novel functional food product.

1. INTRODUCTION

Activity of microorganisms is utilized to produce food and beverages for thousands of years. Food produced with spontaneous fermentation evolves as a result of the activities of complex microbiota. Due to the development of food and nutrition science the potential probiotic strains have got great importance in the selection of industrially microorganisms, to be used in food processing. The most widely used probiotics are found among lactic acid bacteria and bifidobacteria species. Nowadays, nutritional aspects of health protection receive an increasing emphasis. Therefore the demand of consumers for non-dairy based probiotic products like fermented soy-based products, vegetable and fruit juices is increasing. Vegetables and fruits, and their juices have several beneficial effects for human health because they are rich in antioxidants, vitamins, fibres and minerals, and are consumed by a large percentage of the global consumer population. Vegetable and fruit juices may also serve media for cultivating probiotics. The bioactive components may be absorbed even better from juices than from plant tissues (Brandt *et al.*, 2004; Luckow & Delahunty, 2004). In this report carrot, tomato, sour cherry and orange juice are focused on. These juices or drinks are preserved through fermentation with probiotic bacteria: protective and nutritive properties of them are improved (Saarela *et al.*, 2011). Probiotic lactic acid bacteria (*Lactobacillus*, *Streptococcus*) and *Bifidobacterium* species have health-promoting properties by maintaining an improved intestinal bacterial composition, stimulating the immune response, having antimutagenic effect, protecting against infection etc. (Mercenier *et al.*, 2002; Kaur *et al.*, 2002). Among starter cultures *Bifidobacterium lactis* Bb-12 (Chr. Hansen) and *Lactobacillus casei* Shirota (Yakult) are widely used in dairy industry because these strains have numerous physiological and technological advantages. In human studies *B. lactis* Bb-12 has shown efficacy in prevention of traveller's diarrhoea, treatment of rotavirus diarrhoea, modulation of intestinal microbiota, improvement of constipation, modulation of immune response and alleviation of atopic dermatitis symptoms in children (Alander *et al.*, 2001).

The goal of this study was to assess suitability of raw material from plant origin as a substrate for growth of selected probiotic bacteria, and to make trials for preparation of non-dairy products with these strains, because raw materials do not contain dairy allergens that might prevent usage by certain segment of the population (Netzel, 2002; Lavermicocca, 2006).

2. MATERIALS AND METHODS

2.1. Micro-organisms and their maintenance

Table 1: Applied microorganisms and these sources

***Bifidobacterium* strains**	**Source**
B. lactis Bb-12	Chr. Hansen
B. bifidum B3.2	Human isolate
B. longum A4.8	Human isolate
B. longum Bb-46	Chr. Hansen
B bifidum NCFB 1454	National Collection of Food Bacteria

Bifidobacteria (Table 1) were grown anaerobically (in Bugbox anaerobic chamber, Ruskinn Technology) at 37 ºC for 48 hours in TPY medium, that had the following composition (per litre): trypticase (BBL) 10 g, phytone (BBL) 5 g, glucose 5 g, yeast extract (Difco) 2.5 g, Tween 80 1 mL, L-cysteine HCl 0.5 g, K_2HPO_4 2 g, $MgCl_2$ x 6 H_2O 0.5 g, $ZnSO_4$ x 7 H_2O 0.25 g, $CaCl_2$ 0.15 g, $FeCl_3$ 0.03 g, its pH was adjusted to 6.0 and sterilized at 121ºC for 15 min.

Media and raw materials: Fermentation experiments were carried out fruit (orange and sour cherry) and vegetable (tomato and carrot) juice. The different juice was purchased in retail store (Sió, Naturpur).

2.2. Applied methods

Conditions of fermentation: Fermentation was initiated with defined concentration of the relevant *Bifidobacterium* strains. All trials were carried out under anaerobe conditions in Anaerobe Jar+GasPak System (OXOID) or in Bugbox anaerobic chamber at 37°C. Fermentation was followed by counting the colony forming units (CFU) and pH. In some cases the spectrum of organic acids and the quantities of typical acids were also determined.

Determination of colony forming units: Beeren's agar was used to determine the concentration of *Bifidobacteria* (Beeren's, 1990). Serial tenfold dilutions were prepared from the ferment broth. From the suitably diluted samples aliquots were transferred into Petri dishes and mixed with the appropriate medium. After the solidification the plates were incubated under anaerobe conditions in Anaerobe Jar+GasPak System or in Bugbox anaerobic chamber at 37°C. The colonies were counted after 48 or 72 hours incubation.

Determination of organic acids by HPLC: The samples were centrifuged at 14000 rpm for 10 minutes and the supernatants were filtered through 0.45 μm polyvinylidene diflouride membrane (Waters, Milford, MA, USA) before injection. Acetic acid, lactic acid, citric acid, butyric acid, succinic acid, propionic acid, tartaric acid, malic acid content was measured with HPLC equipment with the following technical data: Detector: PDA detector (210 nm); column: Aminex HPX-87H; mobile phase: 5 mM H_2SO_4; flow rate: 0.5 ml/min; column and detector temperature: 45°C.

Determination of antioxidant capacity:
The Blois's method was applied which is based on the binding of DPPH radicals.

3. RESULTS

3.1. Ability of growth in fruit juice

Tested *Bifidobacterium* strains utilized sour cherry and orange juice well, but to varying degree as fermentation medium. By the 24th hour of fermentation cell concentration increased

two orders of magnitude, in some cases three orders of magnitude. The best growth ability was achieved by *B. longum* Bb-46 in orange juice, reaching 0.344 h^{-1} growth rate. Cell count increased three orders of magnitude: from the initial 6.2×10^6 cfu/mL to 1.8×10^9 cfu/mL in the end of the fermentation. The weakest growth activity was shown by the *B. bifidum* NCFB 1454 strain both in sour cherry and orange juice, which is well reflected in the growth rate values (1.08 h^{-1} and 0.14 h^{-1}). Comparing the change of cell concentration in sour cherry and orange juice, it can be stated that the strains had shown better growth ability and metabolic activity in the orange juice. It was demonstrated through the growth rate and generation time (Table 2).

Table 2: Growth rates and generation times of *Bifidobacterium* strains in fermented sour cherry and orange juice

***Bifidobacterium* strains**	**Sour cherry juice**		**Orange juice**	
	μ_{max} (h^{-1})	t_g (h)	μ_{max} (h^{-1})	t_g (h)
A4.8	0.222	3.12	0.266	2.60
Bb-12	0.198	3.50	0.151	4.59
Bb-46	0.257	2.69	0.344	2.01
B3.2	0.167	4.15	0.309	2.24
NCFB1454	0.108	6.40	0.140	4.94

To compare the growth activity of applied strains in different natural media the fermentation process in the vegetable juices were also performed. Results showed that vegetable juices provided better growth media for the tested strains. The best growth activity was shown by the *B. lactis* Bb-12, *B. longum* Bb-46 and *B. longum* A4.8 strains in carrot juice. The highest cell concentrations (8.12×10^8 and 1.25×10^9 cfu/mL) were detected in the 12 to 20 hours range, and the growth rates were between 0.264 and 0.339 h^{-1} (Table 3).

Table 3: Growth rates and generation times of the tested strains in course of the fermentation of carrot and tomato juices

***Bifidobacterium* strains**	**Carrot juice**		**Tomato juice**	
	μ_{max} (h^{-1})	t_g (h)	μ_{max} (h^{-1})	t_g (h)
A4.8	0.339	2.04	0.371	1.87
Bb-12	0.264	2.63	0.151	4.59
Bb-46	0.264	2.63	0.323	2.15
B3.2	0.202	3.43	0.275	2.52
NCFB1454	0.200	3.47	0.234	2.96

3.2. Changes in quantity of organic acids

Final products of the sugar degradation in bifidobacteria are acetic acid and lactic acid, generally in 3:2 ratio, and without CO_2 production. The bifidobacteria can produce small amounts of succinic acid. Butyric acid and propionic acid production is not typical. The largest amount of oxalic acid (3.47 mg/mL) was present during the fermentation of sour cherry juice. Orange juice contained the highest concentration of citric acid (64-65 mg/mL), while in case of the sour cherry juice 21-29 mg/mL were measured. Amount of citric acid did not change in the course of the fermentation in any of the juices. Highest concentration of malic acid (54.3 mg/mL) was detected in sour cherry juice; after the fermentation amount of malate decreased significantly: from 14.29 mg/mL to 17.45 mg/mL in all fermentation. Due to the metabolisms of the strains the concentrations of formic acid, succinic acid, lactic acid and acetic acid increased during the fermentation. Concentrations of propionic and butyric acid did not change.

3.3. Changes in antioxidant capacity of fermentation

Fruits are rich in functional components such as minerals, vitamins, fiber and antioxidant compounds. The selected fruits are rich sources of flavonoid compounds that are bioactive compounds so they have high antioxidant capacities. These arguments have led us to explore the extent to which antioxidant activity changes during the fermentation. In most cases – though slightly, but – the antioxidant capacity decreased in course of fermentation with bifidobacteria (Table 4). Exceptions were if orange juice was fermented with *B. lactis* Bb-12 and *B. longum* A4.8. This result is encouraging because these compounds are able to maintain their functionality in the fermented juice.

Table 4: Changes in antioxidant potential of fermented juice

Products fermented with bifidobacteria	**Antioxidáns potential (mgTE** (trolox ekvivalens)**/mL)**	
	Orange juice	**Sour cherry juice**
Initial value	2.512±0.070	4.675±0.133
A4.8	3.517±0.133	3.536±0.007
Bb-12	2.705±0.105	3.036±0.084
Bb-46	2.601±0.392	2.729±0.210
B3.2	2.650±0.154	3.106±0.182
NCFB1454	2.566±0.105	4.135±0.112

4. DISCUSSION

Our studies directed towards the application of fruit-based raw material for development of probiotic products. The orange juices serve appropriate media for cultivating bifidobacteria. The best growth condition showed the *B. longum* Bb-46 in the fruit juices. This strain is a dairy starter culture with good techno-functional properties. The antioxidant capacity of juices slightly changed during the fermetation. Based on our results the development of probiotic fruit juices may be prognosticated.

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EXTRACELLULAR LIPASE PRODUCTION IN SOLID STATE FERMENTATION USING AGRICULTURAL AND FOOD INDUSTRIAL BY-PRODUCTS AS SUBSTRATES

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SUMMARY

Lipase enzymes hydrolyse triacylglycerols, but under certain conditions, they are able to catalyse the synthesis and translocation of ester linkages. Since filamentous fungal lipases are frequently used in various industrial applications, investigation of their production on cheap and natural substrates has great importance continuously. Zygomycetes fungi are well known as good lipase sources, but there is little information on the enzyme production under solid-state fermentation conditions. Use of agro- and food industrial by-products such as crop and oilseed residues is a low-cost and environmental friendly biotechnological technique for production of lipase enzymes with industrial interest.

In this study, lipase production of some zygomycetes fungi from the genera Rhizopus, Rhizomucor, Mucor, Umbelopsis and Mortierella was tested during solid-state fermentation of various plant residues with high lipid content.

1. INTRODUCTION

Lipase enzymes (E.C. 3.1.1.3) catalyse the hydrolysis and synthesis of ester linkages in different reaction conditions. There is a growing interest on lipase enzymes because of the wide range of their applications in various biotechnological processes, especially in the food-, detergent- and pharmaceutical industry and in the biodiesel production (Sharma, 2001). Lipases are the most versatile catalysts in the field of food biotechnology including synthesis of flavour and aroma esters as well as quality improvement of the food lipid content (Aravindan et al, 2007).

Filamentous fungi are good lipase sources and many of the produced enzymes are frequently used in the food industry (Damaso et al, 2008). Among the zygomycetes fungal group, lipase production of *Rhizomucor* and *Rhizopus* strains is an intensively studied area and some of their enzymes have been isolated and utilised by the industry. However, our knowledge in reference to the enzyme yield during solid-state fermentation is rather limited. Additionally, lipase activity and production of the oleaginous *Mortierella* and *Umbelopsis* strains is poorly characterised from this aspect. Several studies showed that much lower costs and higher enzyme yields can be achieved with solid-state fermentation than submerged fermentation (Castilho et al, 2000; Couto and Sanromán, 2006). Moreover, solid state fermentation is a promising way for extracellular lipase production using fatty acids and triglycerides rich plant residues (Salihu et al, 2012).

In the present work, agro- and food industrial by-products with high lipid content (oat bran, pressed hempen-, line-, poppy-, pumpkin-, red grape seed residues) have been analysed for lipase production. For solid-state fermentation studies, five fungal strains from the genera *Rhizomucor*, *Rhizopus*, *Mucor*, *Umbelopsis* and *Mortierella* were used and addition of mineral salts and olive oil to the ferments were also tested.

2. MATERIALS AND METHODS

2.1. Strains

Rhizomucor miehei (SZMC 11005; SZMC - Szeged Microbiological Collection, Szeged, Hungary), *Rhizopus stolonifer* (SZMC 13609), *Mucor corticolus* (SZMC 12031), *Mortierella*

echinosphaera (SZMC 11251) and *Umbelopsis autotrophica* (SZMC 11276) strains were used for lipase production tests.

2.2. Source of crop and oilseed residues

The tested hempen-, line-, poppy-, pumpkin-, red grape seed residues were generous gift from Solio Ltd. The residues were remained after extraction of the plant-seed oils. The used oat bran is a product of Natura Ltd.

2.3. Solid-state fermentation

To study the effect of different plant residues and conditions on the extracellular lipase production during solid-state fermentation, five grams of substrate were taken in 100-mL Erlenmeyer flasks and moistened with 5 mL distilled water (base medium) or 9.5 mL mineral salt medium (supplemented medium). The supplemented medium contained (% in w/w substrate in distilled water): 0.75% $(NH_4)_2SO_4$, 0.34% NH_2CONH_2, 1.8% NaH_2PO_4, 0.3% KH_2PO_4, 0.045% $MgSO_4$ x $7H_2O$, 0.0375% $CaCl_2$, 1.5% glucose and 1.5% olive oil as previously was optimized for wheat bran based solid-state fermentation (Falony et al, 2006). After sterilization, the flasks were inoculated with a spore suspension containing 10^6 sporangiospores/mL and incubated at optimal temperatures of the strains (*U. autotrophica* 20 °C, *M. corticolus*, *Rh. stolonifer* and *M. echinosphaera* 25 °C and *R. miehei* 37 °C). After one week, the fermented medium was extracted with 30 mL of 0.1 M sodium acetate buffer (pH 6.0) by incubating at 4 °C for 24 h. The extracts were filtrated and then centrifuged at 16.200*g* for 15 min. The resulted clear supernatants were designated as crude extracts and used for lipase activity assay. All fermentation tests were carried out in three independent experiments.

2.3. Lipase activity assay

Lipase activity was determined by measuring the liberation of *p*-nitrophenol during the hydrolysis of *p*-nitrophenyl palmitate (*p*NPP; Sigma) at 405 nm. *p*NPP stock solution (3 mM) was prepared in dimethyl sulfoxide. After addition of equal volume of potassium phosphate buffer (pH 6.8), 50 µL from this buffered solution was given to 50 µL crude extract. Reaction mixtures were incubated for 30 min at 20, 25, or 37 °C depending on the producing isolate. The reaction was stopped by 25 µL of 10% sodium carbonate solutions. Enzyme activities were measured in 96-well microdilution plates using an ASYS Jupiter HD microplate reader (ASYS Hitech). One enzymatic unit was defined as the amount of enzyme that releases 1 µmol of *p*-nitrophenol in 1 minute under the assay conditions. Enzyme activity values were expressed in U/g dry substrate (gds).

3. RESULTS

The tested isolates showed various lipase yields during the fermentation tests. Interestingly, no growth was observed in case of all isolates on red grape seed residue, which may due to the antimicrobial effect of certain grape seed extract (Shrestha et al., 2012). Figure 1 presents the lipase activity yields obtained after fermentation on base and supplemented medium with the other oilseed residues and oat bran. Results showed that these plant residues are potential substrates for lipase production by the investigated fungi. Enzyme yields were variable influenced by the addition of mineral salt solution and olive oil as support. Considerable increasing in the lipase activity could only be detected at fermentation on

hempen seed with *M. echinosphaera*, line seed with *Rh. stolonifer* and line seed and poppy seed with *U. autotrophica* after addition of these supplements to the media.

The highest lipase yield could be detected by *Rhizomucor miehei* on poppy seed base medium (4990.72 U/gds) which is higher than those reported for *Rhizopus homothallicus* and *Penicillium verrucosum* on other low-cost plant derived substrates such as sugar-cane bagasse and soybean bran, respectively (Treichel et al, 2010). It is worth to mention that *R. miehei* lipase is a frequently used fungal enzyme in many industrial processes (Divakar and Manohar, 2007). The poppy seed residue also proved to be promising substrate for lipase production by *M. corticolus* exhibiting 1482.15 U/gds enzyme activity.

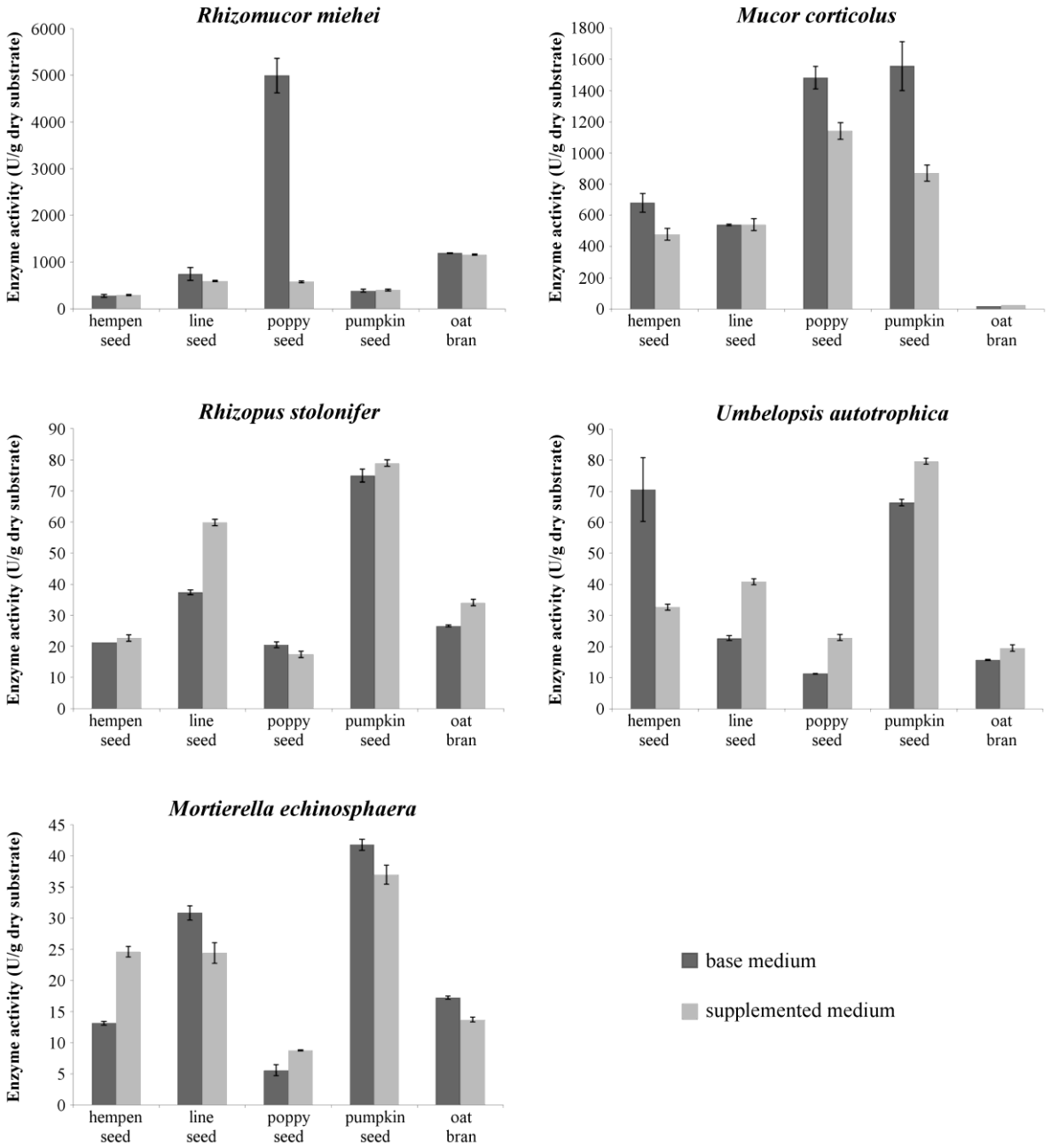

Figure 1: Lipase activities of the tested zygomycetes fungi on different substrates moisturised with only distilled water (base medium) or mineral salt solution (supplemented medium). The presented activity values are averages of three independent measurements performed at the 7th day of the cultivation. Error bars indicate standard deviation.

Except for *R. miehei*, the pumpkin seed was the best substrate for lipase production providing enzyme activities of 1556.79, 78.95, 41.79 and 79.66 U/gds by *M. corticolus*, *Rh. stolonifer*, *M. echinosphaera* and *U. autotrophica*, respectively. It should be noted that the poppy-, and pumpkin seed have the highest lipid content among the tested substrates (11-12% fat content).

In case of oat bran based fermentations, lower *p*NPP hydrolysis could generally be detected comparing to that achieved on most oilseed residues; however, this substrate was the one of the best inductors for lipase production by *R. miehei* (Figure 1).

4. CONCLUSIONS

Many of agro-industrial by-products as substrates can potentially be applied for lipase production (Treichel et al, 2010). In this study, we tested the lipase production of five zygomycetous fungi on hempen-, line-, poppy-, pumpkin-, red grape seed residues and oat bran. Except red grape seed residues, it is proven that these substrates are applicable for high-yield lipase production by the investigated isolates. It is also revealed that addition of mineral salts and olive oil may increase the enzyme yield by certain substrates which, however, may be different by each isolates.

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EFFECTS OF ACIDS AND YEAST STRAINS ON ALCOHOLIC FERMENTATION OF BOSC KOBAK PEAR MASH

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SUMMARY

The aim of this study was to determine the effects of commonly used yeasts and acids on the pálinka, which is a Hungarian spirit fruits. In this study Bosch kobak (Pyrus communis) pear was used for broth, three Sacharomyces cerevisiae yeast strains (YS4, 342, 228) with different fermentation characteristics was applied, pH was set with sulphuric, phosphoric and lactic acids. Beside general analytical methods HPLC technique was used to analyze the fermentation broth. Most rapid fermentation occured with the S. cerevisiae strain 342 paired with sulphuric acid. Gas chromatography methods were used to detect major volatile components. PCA analyzis of major volatile components of distillates show that phosphoric acid and sulphuric acid have similar effects on the distillates, lactic acid regulated broth results a different character of distillate. Yeast strain YS4 paired with sulpuric acid resulted a unique group of distillate character showing no similarities to any other distillates.

1. INTRODUCTION

Pálinka is Hungary's national spirit and a so called Hungaricum. This should be made of fruits produced in Hungary using double-distilled technology. Generally, pálinka making technology starts with mashing and continues with alcoholic fermentation using Saccharomyces cerevisiae, thus quality of the product highly depends on various factors such as quality of fruits, applied yeasts, fermentation conditions, supplemented materials (acids, enzymes etc.). Sacharomyces cerevisiae strain 342 is widely used in distilling industry, so it is also a quite popular strain in the pálinka making sector in Hungary just like strain 228 which was took over from the wine industry, strain YS4 makes a cheap yeast it is used in the baking industry. In recent research, our goal was to analyze the effects of various yeast strains and acid types on the alcoholic fermentation of the Bosc kobak pear.

2. MATERIALS AND METHODS

Fruits, chemicals and microorganism: Bosc koback pear was purchased from local store of Goncsej region. Phosphoric, sulphuric and lactic acids were from VWR International Co. Three different Sacharomyces cerevisiae strains were applied, strains 342 and 228 were delivered by the Kokoferm Ltd., Gyongyos, the other was a baker yeast, YS4 bought from a local store.

Mashing and fermentation: Pear was washed by tap water before mashing which consisted of slicing and disintegrating. The broth then was divided and transferred into different 500 mL flasks with about 400 ml broth/each. Before alcoholic fermentation, the pHs of mashes were adjusted to pH 3.0 using phosphoric, sulphuric and lactic acids separately. Then 0.1975g Uva-Vital yeast nutrient and 0.0197g pectolitic enzyme were added in each flask. Fermentation was started by adding pretreated yeast preparation and performed at 16 °C for 7 days. Samples were taken in one day period and analyzed by different methods.
General analytical methods: sugar content in mash was monitored by refractometriab (BRIX%), reducing sugar (BCA method, Rezessy et al., 2007), HPLC. Titrable and volatile acidity were determined using titration technique with 0.1N NaOH.

HPLC technique: Qualitative and quantitative analysis of saccharides and ethanol, methanol was performed with a Surveyor HPLC instrument manufactured by Thermo Scientific Corporation. The instrument consists of a 4 channel Surveyeor pump, an automatic Surveyor sampler, a Surveyor RI and PDA detector, a stainless steel Bio-Rad Aminex HPX-87H column, (300 x 7.8 mm i.d. packed with 9 µm particle size, 8% cross linkage, pH range 1–3) was applied. Running time was 20 minutes, mobile phase 0,005n H_2SO_4 delivered in isocratic mode at 0.5ml/min flow rate. Detector and column temperature was both set to 45°C. The automatic sampler was set to 5µl.

GC analysis: After 7 days of the fermentation, samples were distilled using Gibertini DEEPV lab distiller unit. Volatile components of the distillates were determined with GC-FID. Principal component analysis (PCA) was used for analysis of the distillate-s's identified components.

3. RESULTS AND DISCUSSION

All three *S. cerevisiae* strains performed rapid fermentation in the case of the sulphuric acid, while the opposite effect was observed the case of the phosphoric acid. Samples treated with lactic acid showed intensive fermentation at every yeast strains, sulphuric acid treatment resulted in slow fermentation but also showed the highest reducing sugar consumption and the higher alcohol content. Ethanol concentration was measured to be higher at the sulphuric acid samples. Weight losses throught CO_2 formation also indicate the intensive fermentation. The capability of utilization of sugars was demonstrated in Table 1. Regarding the above mentioned results the best acid appears to be the sulphuric acid. Similar performance was detected in all cases of using yeasts.

Table 2: Amount of sugar utilized by yeasts

	YS4			228			342		
Sugar consumption	**C3H6O3**	**H2SO4**	**H3PO4**	**C3H6O3**	**H2SO4**	**H3PO4**	**C3H6O3**	**H2SO4**	**H3PO4**
Day1	1,025	2,6	2,35	1,475	2,625	2,45	0,825	2,675	2,625
Day2	5,1	4,575	2,625	4,975	4,075	2,825	3,675	3,9	2,55
Day3	1,5	0,375	2,6	1,075	0,775	2,225	2,65	0,975	1,975
Day4	0,075	0,1	0,575	0,05	0,1	0,375	0,1	0,175	0,725
Day5	0,05	0,05	0,25	0,05	0,05	0,2	0	0	0,175
Day6	0,025	0,05	0,1	0	0,1	0,15	0	0	0,15
Day7	0,125	0,125	0,075	0,05	0,1	0,05	0,025	0	0,075
Sum	7,9	7,925	8,725	7,675	7,975	8,275	7,275	7,8	8,3

The pH of the fresh grout fruit was 4.26 and the pH of mash was adjusted to pH 3 (+/- 0.08) at the beginning of the fermentation. During the fermentation process minimal changes of the pH of mashes were measured (Figure 1). Similar results were obtained in the case of titrate acidity.

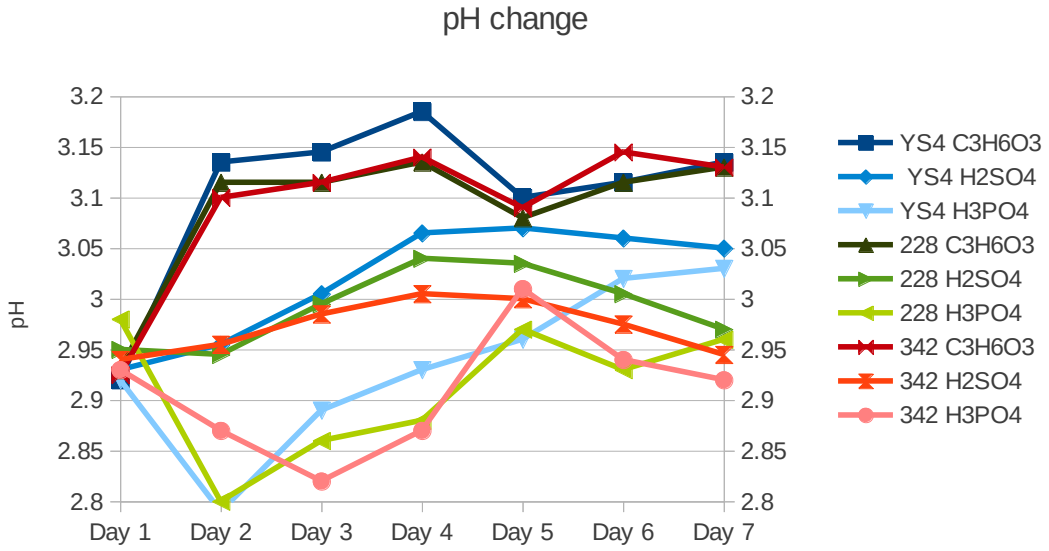

Figure 12: pH changing dynamics during the fermentation

Volatiles of fermented mashes were determined by GC and analyesed using Principle Components Analysis method (PCA). Results of nine experimental runs are demonstrated in Figure 2. It can seen that results of lactic acid treatment were significantly distinct from two other acids. Stain 228 and 342 appear to have many similarities when the pH is set with phosphoric acid or sulphuric acid. Baker yeast, YS4 paired with phosphoric acid fell apart from the other distillates.

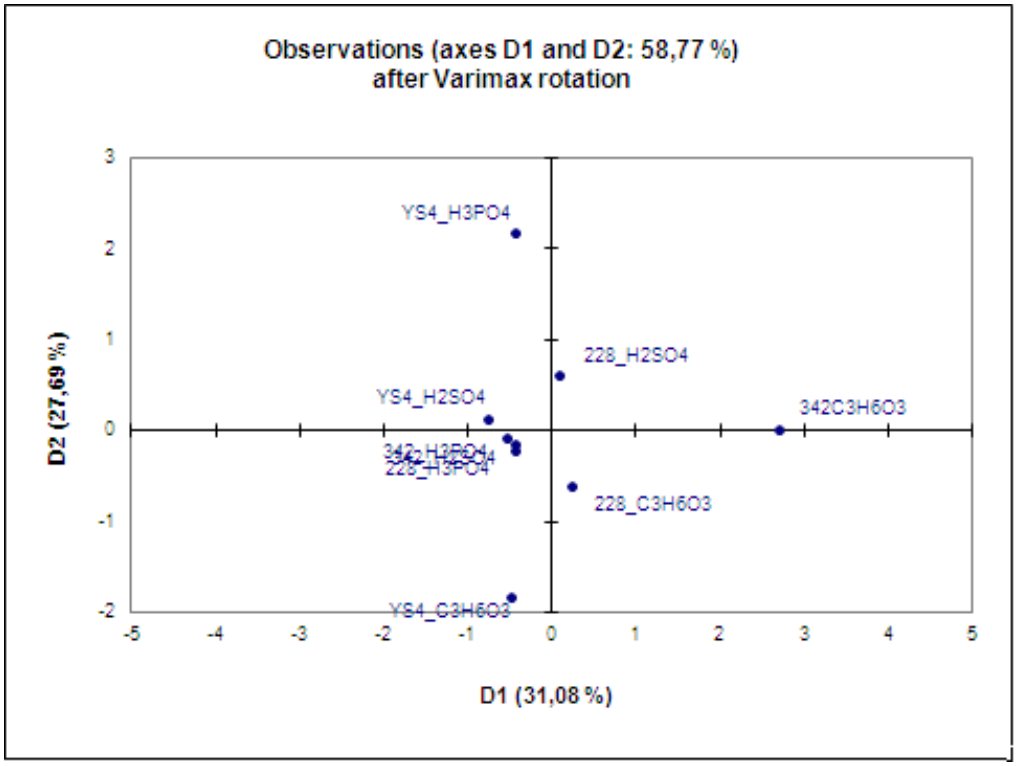

Figure 2: PCA analysis of major volatile components

4. CONCLUSION

Cobak Bosch pear was good fruit for production of palinka with rich in volatile components. Adjusting pH of mash with lactic acid was the best choice in both fermentation process and aroma profile. More studies are needed to develop suitable technology for production of standard quality of palinka.

ACKNOWLEDGEMENT: *This work is supported by National Development Agency (Project no. TÁMOP-4.2.1./B-09/1-KMR-2010-0005, TÁMOP-4.2.2/B-10/1-2010-0023 and TECH_09-A3-2009-0194), as well as Bolyai Research Grant from Hungarian Academic of Sciences.*

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THE EFFECT OF A MICROWAVE TREATMENT TO THE ALCOHOLIC FERMENTATION AND DESTILLATION

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SUMMARY

The alcoholic fermentation is a well-known process but it is impressable and adjustable by changing of many factors. We completed with a microwave pretreatment the controlled fermentation technology. We compare the feature of the fermentation of normal fermentated mash and the microwave pretreatmented mash.
We use the non-thermal effects of the electromagnetic radiation and we influence the life activities of microorganism and enzymes in the mash by this.
The positive effects of this method:

- reducing the time of the fermentation
- growing the volume of the final product (ethanol)

OPTIMISATION OF CELLULASE PRODUCTION OF SELECTED FILAMENTOUS FUNGAL STRAINS

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SUMMARY
Cellulase activity of nine previously identified filamentous fungal strains isolated from agricultural wastes and compost was characterised. The Trichoderma hamatum SZMC 1641 and Humicola insolens K41-EF3 isolates, which showed the highest enzyme activity, were chosen for further studies. The optimisation of the physical and chemical parameters for enzyme production was carried out by changing the incubation time, temperature and composition of the induction media. The results showed that the cultures reached the maximum cellulase activity after 3-4 days and the optimum temperature for cellulase production was 30°C, however in case of H. insolens incubation at 37°C yielded higher β-glucosidase activity. Optimisation experiments performed by changing the carbon sources led to two-threefold increase in enzyme activity of T. hamatum SZMC 1641 and H. insolens K41-EF3.

1. INTRODUCTION

Cellulose is the most abundant renewable resource produced in the biosphere and it serves as an important raw material for many industries (Zhang et al., 2006). The enzymatic hydrolysis of cellulose is the key step of these techniques. The multi-component enzyme "cellulase" degrades cellulose by synergistic action of different enzymes: endoglucanases, exoglucanases and β-glucosidases (Matkar et al., 2013). Many factors can influence the production and activity of these enzymes, such as temperature, pH, cations, carbon and nitrogen sources, and surfactants (Dashtban et al., 2011; Dhillon et al., 2012; Matkar et al., 2013).

The most prominent representatives of the cellulolytic microorganisms are filamentous fungi, as they can produce high amounts of extracellular cellulases and hemicellulases (Dhillon et al., 2012). Actually, the species that are used frequently for production of commercial cellulases belong to the genera *Trichoderma* and *Aspergillus* (Zhang et al., 2006). The aim of the present study was to select cellulolytic strains with the highest enzyme activity from isolates originated from agricultural waste and compost, and to optimise their enzyme production.

2. MATERIALS AND METHODS

2.1. Filamentous fungal strains

The following filamentous fungal strains were tested: *Absidia corymbifera* TMP3, K3-EF1; *Chaetomium sp.* EFP5; *Humicola insolens* K41-EF3, K43-EF3; *Rhyzopus orizae* SzP2; *Trichoderma atroviridae* T66; *T. hamatum* SZMC 1641 and *T. reesei* F651. These isolates were grown at 30°C and maintained in Complete Medium (**CM**: 20 ml 5x Czapek solution; 10 µl Vogel microelement solution; 0.3 g yeast extract; 0.3 g peptone; 0.3 g peptone from Soja; 2 g glucose; 1.5 g agar; final volume was adjusted to 100 ml with distilled water).

2.2. Induction of the cellulase production and the enzyme activity assays

To induce the cellulase production of the strains cellulose and carboxymethyl cellulose (CMC) were used as substrates. The mycelia were produced by growing the fungal strains in

25 ml of CM broth (CM without agar). The CM broth was inoculated by $2.5x10^6$/ml conidia and incubated with shacking (140 rpm) at 30°C. The mycelia were collected by centrifugation (10 minutes, 4000 rpm), washed twice with sterile distilled water, and used to inoculate (inoculum concentration was 4%) the induction media: CMC or Cellulose broth (1% mannitol; 0.5% KH_2PO_4; 0.2% $NaNO_3$; 0.1% $MgSO_4x7H_2O$ and 1% substrate).

The fungal cultures were incubated at 30°C for the appropriate time interval and cell-free supernatants of the cultures were used for further assays. To detect the cellulase secretion of the isolates 20 µl cell-free supernatant was dropped onto CMC agar (1% high viscosity CMC and 1.7% agar). Plates were incubated for 1 hour at 37°C and 50°C, respectively. Cellulase (endoglucanase) activity was detected by Congo red staining where the clearing zone indicated the digestion of the substrate (Sazci et al., 1986).

The total cellulolytic activity of the strains was determined by measuring the reducing sugar content of the samples. The Dinitrosalicylic Acid (DNS) Colorimetric Method (Miller, 1959) was applied. The reducing sugar content was measured directly from the cell-free supernatant, and at the same time the enzyme activities were determined by adding 1% cellulose powder to 5 ml supernatant, incubated for 2 hours at 30°C, and produced reducing sugars were measured. The enzyme unit was calculated as follows: Unit = [reducing sugar concentration (mg/ml)/reaction time (h)].

The activity of the β-glucosidase was determined by GOD/PAP method where the instructions of the manufacturer (Diachem Ltd, Hungary) were followed.

2.3. Optimisation of cellulase production

Different chemical and physical conditions (time period of incubation in the inducer media, temperature of incubation, availability of C-sources) were studied to improve and optimise the production of cellulases of the selected filamentous fungal strains. The CM broth used for inoculum production varied in the carbon sources as follows: 2% glucose; 1% glucose; 1% lactose. The 1% cellulose containing induction medium was modified as well: in these experiments it was also applied without mannitol supplementation.

3. RESULTS AND DISCUSSION

3.1. Screening for cellulolytic strains

For testing the cellulolytic ability of the filamentous fungal strains the enzyme secretion was promoted by adding CMC to the media, and the substrate digestion was examined by Congo red staining. Only three out of the nine tested strains, *A. corymbifera* TMP3, *A. corymbifera* K3-EF1 and *Chaetomium sp.* EFP5, gave positive results, but if the total enzyme activity of the strains was determined all isolates proved to be cellulolytic. In this case *T. hamatum* SZMC 1641, *T. reesei* F651, *H. insolens* K41-EF3 and *A. corymbifera* TMP3 showed the highest cellulolytic activities. On the basis of these results the following five fungal strains were selected for further analyses: *A. corymbifera* K3-EF1; *T. hamatum* SZMC 1641; *H. insolens* K41-EF3; *Chaetomium sp.* EFP5 and *T .reesei* F651.

In the next screening steps the substrates as inducers for cellulase production were examined. Both CMC and cellulose could induce the enzyme production of the strains except of *T. reesei* F651, which was positive only in case of CMC. The enzyme activities were checked at 37°C and 50°C, and it was established that the secreted cellulases were active at both temperatures.

The reducing sugar content of the two- and five-day-old cultures were also determined. The highest concentration was measured in case of *Chaetomium sp.* EFP5 which was followed by *T. hamatum* SZMC 1641 and *H. insolens* K41-EF3 strains. For optimisation

experiment SZMC 1641 and K41-EF3 were chosen because of the very poor growth rate of the *Chaetomium sp.* EFP5 isolate.

3.2. Optimisation experiments

3.2.1. Effect of incubation time on cellulase production

To determine the optimum incubation time for cellulase production the enzyme secretion of the strains was followed for seven days. In case of *H. insolens* the maximal reducing sugar concentration was measured at the third and fourth days, while *T. hamatum* reached the maximum at the second day, however the measured reducing sugar concentrations were lower than in the case of *H. insolens.* These findings can be explained by the fact that the fungi assimilate the released sugars and *T. hamatum* might utilise all the released sugars for its growing. After the third day this strain maintained a constant enzyme activity, as it is shown in Figure 1.

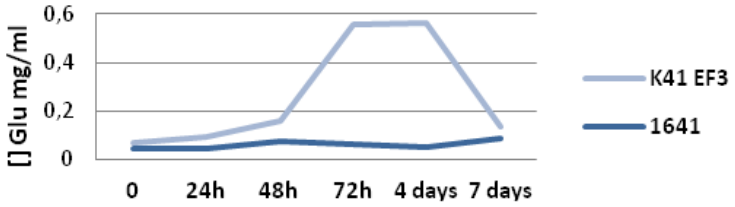

Figure 1.: The effect of the incubation time on the cellulase activity of *H. insolens* K41-EF3 and *T. hamatum* SZMC 1641

3.2.2. Effect of temperature on cellulase production

Temperature influences the physiology and metabolism of the organisms. It is known that *H. insolens* is a thermotolerant, while *T. hamatum* is a mesophilic species. The cellulase production could highly depend on incubation temperature, thus the enzyme fermentation experiments were carried out at 30°C and 37°C.

As it was expected *T. hamatum* could not grow and produce enzyme at 37°C. *H. insolens* showed higher β-glucosidase activity at 37°C, while there were not significant differences in total enzymatic activities compared to the two different temperatures.

3.2.3. Effect of carbon sources on the cellulase production

The various carbon sources such as lactose, galactose, cellobiose, mannitol and cellulose can act as inducers for cellulases production. The application of these substrates could result in increased enzyme production (Dashtban et al., 2011; Dhillon et al., 2012).
In our experiments glucose and lactose were used as carbon sources for inoculum production, and cellulose for induction of cellulase secretion. The effect of mannitol on cellulase was also studied.

If the lactose was used for inoculum production the cellulolytic activity of *T. hamatum* was significantly increased, in particular of the β-glucosidase. In case of *H. insolens* the media with 2% glucose gave the best results for inoculum production and enzyme activity. Mannitol generally repressed the cellulolytic enzymes. The highest enzymatic activity was measured in case of *T. hamatum* when mycelia were produced on 1% lactose, and the induction media did not contain mannitol, while in case of *H. insolens* the combination of 2% glucose in inoculum media and absence of mannitol in induction media proved to be the best (Figure 2).

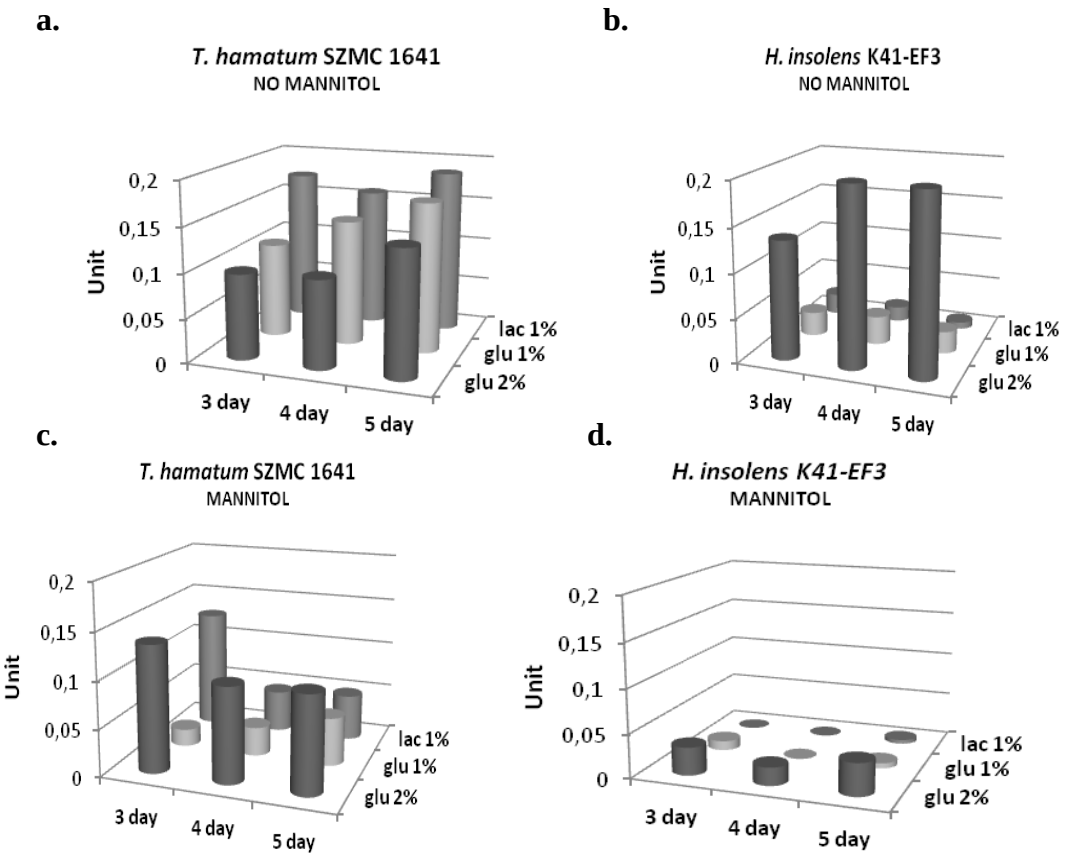

Figure 2.: Total cellulolytic activities of the strains in cellulose media without mannitol (**a.**, **b.**) and supplemented with mannitol (**c.**, **d.**);. Inoculum cultures were produced in CM media containing glucose (glu 2%, glu 1%) or lactose (lac 1%)

4. CONCLUSION

By carrying out the experiments two-threefold increased enzyme activity was realised in case of *T. hamatum* SZMC 1641 and *H. insolens* K41-EF3 strains, but further optimisation studies are needed to investigate the effects influencing the cellulase secretion and activity and to improve enzyme production by these fungi.

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THE EFFECT OF KEEPING THE LEES TO WINE POLYPHENOL COMPOSITION

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SUMMARY
Due to autolysis of the yeast the composition within the nitrogen-containing substances, concentration of the amino acids altered in keeping lees. Previous studies has been shown that the yeast can reduce the concentration of polyphenol. This paper examined the effect of keeping lees in Chardonnay and Sauvignon Blanc wines. Study focused on changes in the polyphenol composition, furthermore the composition of simple phenols, also stood within the Tyrosol. Measurement results have shown that the yeast reduce the amount of polyphenols and increased amino acid concentrations in the names of tyrosine residues resulting amount is Tyrosol.

1. INTRODUCTION

The development of the wine maturation of a very complex process that the fermentation to bottling, and even extends to the final costumer. The duration of the conversion feature of the ongoing transformation which results in the formation and increasingly become final wine aroma, flavor, character. Wine is the clarity grade of a lot of lives over the course of the development and management in. The new wines heavier sediment material based on the density difference in the wine tanks, barrels made a total bottom sediment dense dregs (Eperjesi et. al., 2000).

The sur lie aging that is in keeping lees, dregs usually circulate. This will enhance the body are in the wine, reduced the catchy of increased complexity in red wine aroma and flavor, fragrance length. The practice, known as a sweeping move, mixing up which battonage other words, it has traditionally been connected with the technique. The battonage promotes homogenization of the composition and facilitates the wine better mixing of the entire quantity of lees. It has been shown that the lees resuspension by agitation during maturation of the macromolecular extract significantly increases the wine.

Many low-weight compound is soluble in autolysis of microorganisms in the wine such as nitrogen compounds, some of the polysaccharide and lipid. Thus, the wine is matured on lees noticeable effect on the composition of the product which (González- Marco, Ancin-Azpilicueta, 2006). evious studies have shown that the yeast polysaccharides vary by tribe. Result in the case of red wines lees aging-based interactions between polysaccharides and polyphenols. The migration of the polysaccharides, the yeast strain, turbidity during the fermentation time, the contact time and temperature also affects (FUSTER, ESCOT, 2002).

The effect of aging lees mainly studied in the nitrogen-containing compounds (GONZÁLEZ- MARCO, ANCIN- AZPILICUETA, 2006; GONI, AZPILICUETA, 2001) and subsequently the red wine polyphenol composition regards as well (CHARPENITER, FEUILLAT, 2008). Polyphenols are very important compounds that have antioxidant and organoleptic properties of the wine greatly influenced by it. can be found in grapes, grape must and wine as well (KENNEDY et. al., 2006). Prone to oxidation, browning associated spin-offs and other agents, for their presence in wine red wines of character design is very important (EPERJESI et. al., 2000). The red wines taste and aroma training, flavonoids, phenols play an important role, while in contrast with the case of non-flavonoid phenols in white wines, the higher phenolic group (LŐRINCZ ET. AL., 1998). The flavonoid concretation of white wines are most of catechins (flav-3-ol) and leukoantociacnins (3,4 diol) (CARO et. al., 2010). However, these compounds carry a bitter taste impression and their presence is only a limited amount of desirable. The wines may increase the catchy taste due to the wooden barrels, as then

hydrolysable tannins soulabe in the wine. The cinnamic aldehyde and benzaldehyde derivatives which extracted from oak barrels also contribute to the bitter sense of taste from the non-flavonoid phenols (KÁLLAY, NYITRAINÉ-SÁRDY, 2008). Most of the phenolic acid like. Caftaric acid in the wine is present in a small quantity that traces out (MONAGAS et. al., 2006).

2. MATERIALS AND METHODS

Experimental design

Sample pair A

The collected grape samples were crushed and pressed, and then we treated the must generated by this process.

Must

stumming: with a 50 mg/L portion of sulphurous acid

enzyme dosage: commercial enzyme – Zimaclar (2 g/hL).

cooling and settling: for 1 day, at 10 °C

After the must handling, we commenced the alcoholic fermentation as described below: nutrient salt dosage: V active premium 24 g/hL at the beginning and in the middle of the fermentation 15 g/hL, too yeast dosage: Premium Chardonnay (20 g/hL)

Sample pair B:

The processing syllabus is almost identical to the syllabus of sample pair A. The only difference is that we also added a certain amount of *sur lie* enzyme: 2 g/hL pectin-splitting enzyme.

Sample pair C:

In this case we added 100 g/hL of "L.P.A. active yeast" in paste and 0.5 g/hL beta-glucanase enzyme to the sample

Determination of the polyphenolic composition.

determination of the total polyphenolic content, applying the Folin–Ciocalteau reagent, calibrated to gallus acid, (KÁLLAY – TÖRÖK, 1999)

the quantity of leucoantocyanins, after heating with a 40:60 compound of hydrochloric acid-butanol containing iron (II) sulphate, spectrophotometrically (FLANZY et al. 1969, modified)

the anthocyanin content measured spectrophotometrically at 550 nm with dilution 2 V/V concentration of 96% ethanol and HCl (FLANZY et al. 1970, modified)

the catechin content in wine diluted by alcohol, at its reaction to vanillin-sulphuric acid, at 500 nm, spectrophotometrically (REBELEIN, 1965)

TAS-value. The determination of the TAS-value was measured with the help of the Randox-test applied in the medical practice (KÁLLAY, TÖRÖK, 1999)

3. RESULTS

Each measurement result is based on a double repetition, thus our results represent average values. The bar graphs shown only the results of the stirring samples as in the sensory analysis of sample pairs of these items higher scores were achieved. The total polyphenol concentration of each sample was lower than the control ones. This is due to the interaction of polyphenols to the yeast. Treatments include a very great difference. Substantial difference can not be detected in the first two months by the effect of lees-keeping. However, the total polyphenol concentration in the second and third month show a significant difference in each sample (Figure 1.).

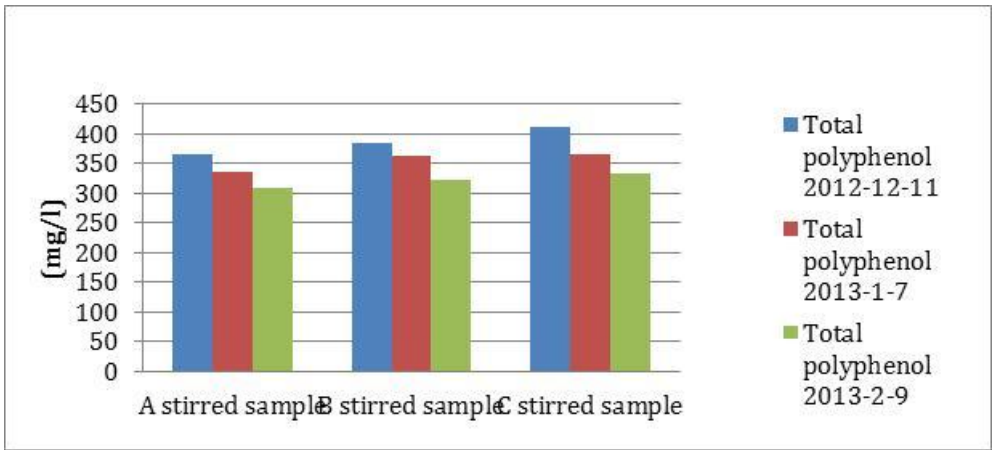

Figure 1: Total polyphenol content

We illustrated the leucoantocyanin content in Figure 2. As with the total polyphenolic content, we were able to observe a decreasing tendency in each wine that was kept on fine lees. There is a significant difference between the stirred versions of the same samples, i.e., the different enzyme preparations influenced leukoantocianin content. (F=115,46 p=0,05, F*=3,49)

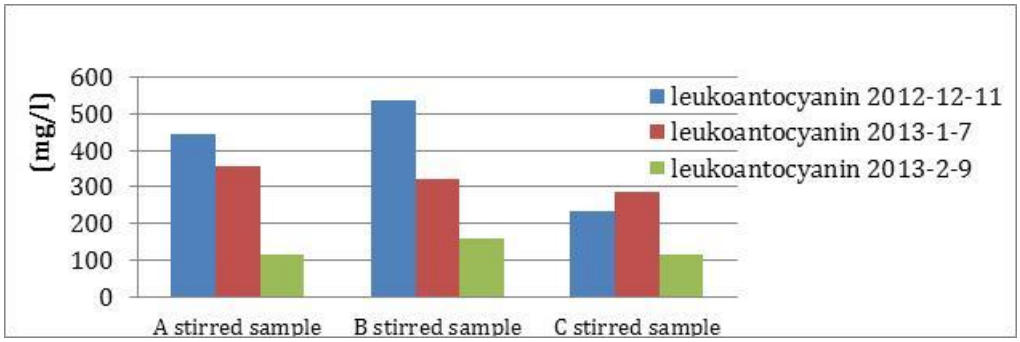

Figure 2: The change of leucoantocyanin concentration

In this case, the influence of yeasts is obvious, as the catechin content is far less than in the control sample (Figure 3.). At the significance level of 95%, no substantial differences can be detected among the samples and not stirred items. (F=225,16 p=0,05, F*=3,49) In line with the change in the total polyphenolic content, we were able to observe the same tendency in regards to each item (Figure 4.). The reason for our findings is that the TAS-value is greatly affected by the total polyphenolic concentration. (F=37,7 p=0,05, F*=3,49)

4. CLONCUSIONS

It is clear from the test results that the lees-keeping a significant impact on the polyphenolic composition of wines. Our analyzes established that the yeast during in the lees-keeping, acts as a flocculant to reduce the total polyphenol concentration. In our experiments, no significant difference could be detected between the effects of certain enzyme preparations, thus, the enzyme is used significantly affect the polyphenolic composition of wines over the lees-keeping. A clear correlation couldn't be detected at concentrations of catechin.

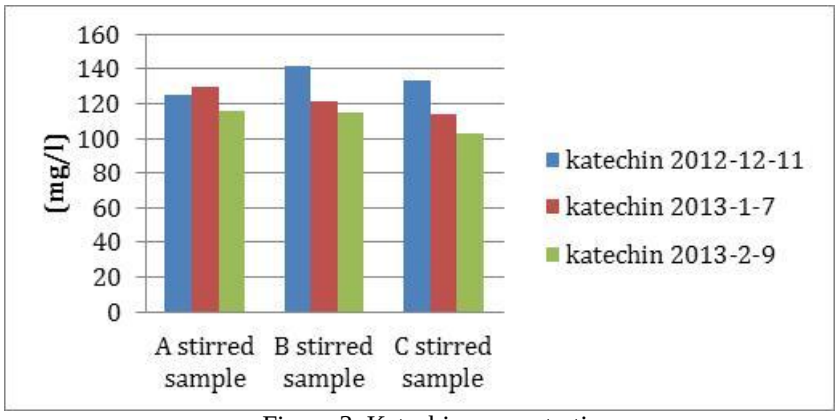

Figure 3: Katechin concentration

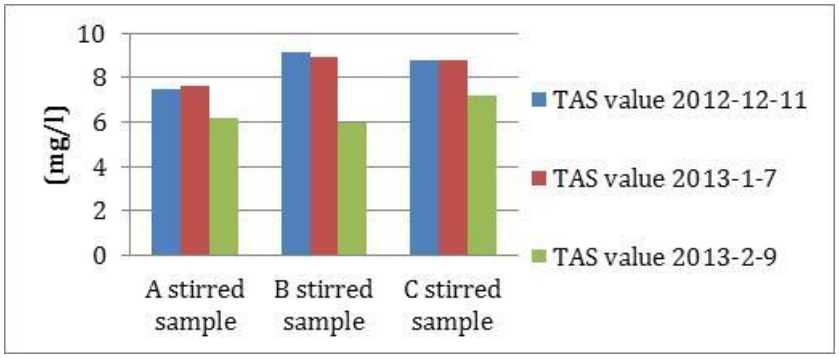

Figure 4: The change of the TAS-value

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EXAMINATION OF THE EFFECTS OF EXTRINSIC PARAMETERS IN POINT OF THE PRODUCTION OF INULINASE ENZYME BY THERMOPHILIC FUNGUS

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SUMMARY

Inulinases are ubiquitously used enzymes for producing high fructose syrup (full hydrolysis by exo-inulinase) or inulo-oligosaccharides (partial hydrolysis by endo-inulinase) in the industry. Inulinase preparations are commercially available nevertheless all of them are from mesophilic *Aspergillus*. In our present work examination of extracellular inulinase from *Thermomyces lanuginosus* was focused. As a result of an earlier selection procedure IMI 140524 strain was found to be the most promising one to produce inulinase. The effects of some extrinsic parameters were examined, furthermore fermentation medium optimisation by Response Surface Method (RSM) was carried out in shaken flasks regarding the pH and the rate of the net and total volume and regarding the pH and the quantity of the injected inoculum. The obtained results contribute to further purification measurements of the inulinase enzyme from the themophilic fungus.


SECTION 2: Food chemistry, analytical chemistry, food microbiology


SHORT TERM SEED GERMINATION – A NOVEL TECHNOLOGY INNOVATION OF A FOOD ADDITIVE

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SUMMARY

Chemical composition and availability of main components as well as the level of bioactive constituents of seed can be radically changed during different periods of life cycles in plant materials. Our technology innovation improves the nutritional and functional properties of soybean by a strictly controlled, short-term germination. Thus we developed a novel product (called YASO), which can be defined as an analogue of meat from compositional point of view. The operation steps of the applied unit allow to eliminate the antinutritive factors, to improve the digestibility and availability of proteins, to reduce the soluble carbohydrate content and energy level, to enhance the concentration of the real active forms of bioactive components (e.g. isoflavons, phenolic acids) and to decrease the level of allergens. The innovated novel material can be used as new additive as it is, and/or can be applied in different food systems (meat, bakery, pasta, extruded, confectionary, sauce, pasty etc. products), as well as in food products having targeted functional properties (reduced energy level, gluten-free, dietetic, vegetarian etc. products).

1. INTRODUCTION

Recently more and more new food is innovated in the industry to ensure appropriate amount and high quality foodstuffs because the population of the world is continuously growing and the eating habits and the lifestyle of the people have changed. The diets are often unbalanced in most of the nations because of the rushing lifestyle and dietary fashion in the developed countries, and also because of the lack of some nutrients – for example the lack of the protein – in the developing countries. Furthermore the number of people who suffer in diseases requiring a special diet is also continuously growing. Therefore our aim is to develop a raw material which is beneficial for most of the above mentioned aspects.

The germination of plants offers a lot of possibilities to increase the nutritional value of the produced foods. Germs are used long ago in the human nutrition for their valuable composition. They have higher nutritional value than the full-grown plants and they contain the nutrients in an easily digestible form.

For our development the soybean was chosen which is prominent compared to other legumes. It contains relatively high amount of proteins and relatively low amount of carbohydrates. One of its biggest advantages is that it is a complete protein source. It‘s further advantageous properties are that it is rich in vitamins and in minerals as well as it has high amount of multi-unsaturated fatty acid. The nutritional and functional properties of soybean seed were modified by a strictly controlled, short-term germination process.

2. RESULTS AND DISCUSSION

Germination is a period of the vegetative part of the plant life cycle. The first step of the biological process is the water intake. At the same time the delivery and the mobilization of the reserved nutrients (proteins, carbohydrates and lipids) are initiated, later that the embryo starts to grow. Germination is a heterotrophic process. The metabolic processes are getting faster, the enzyme activity is increasing more intensively and the operation of the plant hormones is getting more active during germination. In the later phase the autotrophic nutrition will be typical for that plant which already has green parts and so it is able to photosynthesize (Horváth, 2004). The recognition of the importance of the heterotrophic processes led to our innovation; the valuable nutrients have only mild and from nutritional

point of view positive changes. In addition the valuable nutrients can be used to produce healthy food products.

2.1. Physiology of germination

The low moisture content (5-15%) and the inactivity of the metabolic processes are typical for the sound seed (Obroucheva, 1997), hydration and oxygen uptake is necessary to continue the metabolic processes therefore the germination begins with water intake. The last step of the germination is the beginning of the embryonic axis elongation. At this time the first visible sign of the germination (the radicals) appears (Bewley, 1994). Two main phase of the germination can be distinguished. In the first phase swelling of seed and reactivation of germ can be observed. The second period is considered as the mobilization of the reserve nutrients. The major cellular and metabolic processes of germination are shown in Figure 1.

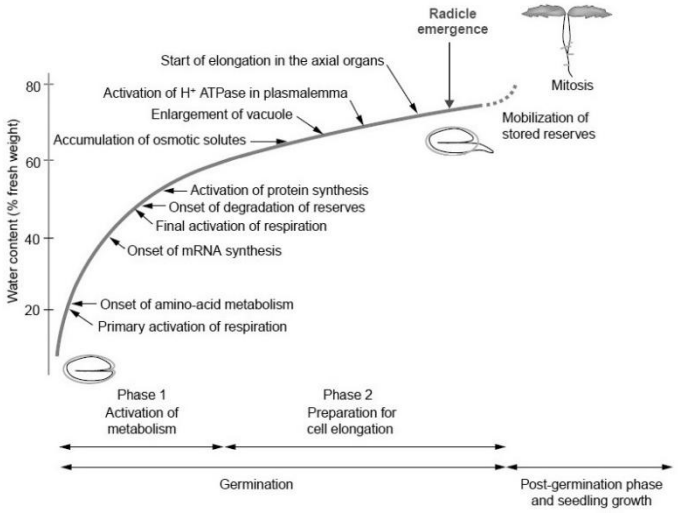

Figure 1.: The main events occurring during germination (Bove, 2002)

The remarkable water intake i.e. the rapid swelling starts the basic metabolic processes. The macromolecules, cell compartments, hormones and enzymes are reactivated in the seed. In this initial phase the water intake is carried out by a physical path and the metabolic pathways are activated step by step. The respiration is getting more and more intensive so the oxygen intake is increasing rapidly. The glycolysis, citric acid cycle and pentose-phosphate cycle are activated. Initially the free amino acids and saccharides are digested and the poorly degradable starches and proteins are digested and used only much later. It is typical for the new macromolecules synthesis that at first the mRNA synthesis initiated, it is followed by the protein synthesis – also the enzyme protein synthesis– and relatively later the DNA synthesis. The second phase of germination begins when the level of hydration exceed the 60%. At this point the water intake slows down and the cells begin to grow towards the embryonic axes. Osmotically active substances are accumulating e.g. saccharides, amino acids and potassium ions. Then the *de novo* synthesis of the hydrolytic enzymes (α-amylases, proteases, ribonucleases, etc.) starts (Salgó, 1986), these enzymes penetrating the endosperm and start to digest the reserves. By reason of the acidification of the cell wall the bonds between the polymers of the cell wall loosen and disintegrated. Finally the surrounding tissues weaken the embryonic axis elongation and the germination ends. As long as the plant is not able to photosynthesize the reserved nutrients are used (Obroucheva, 1997).

2.2. Short term germination

In the short term germination the biological process is stopped approx. 30-50 hours. Beyond this time the biological process of the germination is significantly continue thus nutrient losses occur. During and thereafter the germ uses the earlier released nutrients for its own growth. Thus internal structure and chemical composition of the seed are changed which means that the biological value of the original soybean is partly converted. Important benefits of short-term germination are the intensive activation of hydrolytic enzymes resulting the breaking of the subunit structure of the proteins and partial mobilization of insoluble carbohydrates. The activated hydrolytic enzymes have different other effects in the seed.

The new food additive is produced by the activation of the own enzyme system of the soybean. After preconditioning the seed are soaked till 1,5 – 2,5 hours in 15-35 °C water. After removing this water the germination is started. It can be realize either in light or without light but oxygen has to be ensure all the time. The germination process is stopped after 48 hour (calculated from the first moisten) or after the appearance of the 5-8 mm long germs by a heat treatment. Thereafter germinated soybeans are cool down and dried (Patent description, 2008).

Substances with anti-nutritive character have already been decomposed during the short-term germination, e.g. substances like the enzyme inhibitors and the stachyose which inhibit the digestion. So the nutritional value and digestibility of the product are increasing. During germination phytases are activated and the amount of the phytic acid is decreasing (Suparmo, 1987). The insoluble carbohydrates partly break to saccharides and dissolve so the carbohydrate (and energy) content of the soybean is decreasing. The amount of isoflavones, β-carotene, vitamins and dietary fiber has already been significantly increased during the initial phase of the germination. (Patent description, 2008; YASO, 2013)

2.3. Soybean

The industrial use of soybean is very extensive (animal feeding, catering and also pharmaceutical, cosmetic and biotechnological applications) due to its high biological value. The composition of the mature soybean is approximately: 38% protein, 35% carbohydrate, 18% lipid, 4% crude fibre and 4,5% minerals on dry basis. Its relative high amount of protein contains of 10% albumin and 90% globulin. Globulins are reserved proteins which are mobilized during the germination process. The amino acid profile of soybean is ideal for the human organism and the plant contains all of the essential amino acids. The soybean contains relative low amount of carbohydrate comparing to other legumes. The bigger part of the carbohydrates is insoluble. This part is decomposing to saccharides during the natural process so the carbohydrate content of the bean is decreasing. The soybean contains mainly unsaturated fatty acids as linoleic acid and oleic acid and it is also reach in linolenic acid compared to other legumes. It contains also a remarkable amount of bioactive components (e.g. flavonoids, fitoestrogens, glucosides, phenolic compounds and lignans), minerals and vitamins (mainly E and B vitamins). On the other hand the soybean contains antinutritive factors (different protease inhibitors, hemagglutinins, stachiose) and allergens as well (Belitz, 1985).

2.4. YASO

Our novel, widely applicable and biologically valuable product – called YASO − combines the benefits of the short term germination and the benefits of the seed soybean. It keeps all of the beneficial properties of the soybean and makes the different constituents better exploitable. During the applied germination and heat treatment majority of the components which cause disadvantageous properties are decreased to a minimum level or totally decomposed. The technology use only non GM soybean as raw material and the product does not contain any additives.

Because of the enhanced proteolysis during germination the subunit structure of proteins is decomposed. The digestibility and availability of proteins (amino acids) are improving by the activated endo- and exoproteases (Barcelos, 2002). The energy content of the product is significantly decreased because approximately 15% soluble carbohydrates are dissolved and leaked, so the relative amount of dietary fibre is increased. Moreover, due to the activation of α-galactosidases, β-galactosidases and α-amylases the structure of dietary fibre is weakened and its solubility is increased. Other enzymes show also intensive activation. The activated phytases which digest the phytic acid which blocks the absorption of some minerals are eliminating. The trypsin inhibitors are fully inactivated. Stachiose is dissolved and leaked out

during germination and heat treatment. The amounts of the particular bioactive components are significantly increasing during germination. For example the seed biosynthesizes phenolic compounds to enhance the ability to protect itself. Among isoflavonoids the ratio of the rapidly absorbed aglucons increases at least 2 times at 25 ˚C in 48 hours. The amounts of the allergens are changing dynamically during physiological cycle. Moreover after 48 hour germination a remarkable part of the originally present allergens are inactivated. YASO is a complete protein source and it is rich in dietary fibers, in omega-3 and omega-6 fatty acids. In addition it contains only a low amount of easily mobilized carbohydrates. Due to its beneficial composition it is a high-quality meal replacement Table 1. shows the composition of YASO regarding to 100 g product.(YASO, 2013; Sandberg, 2002).

Table 1.: Composition of YASO [m/m% / 100g product] (YASO, 2013)

	Moisture [m/m%]	60,76		
	Protein [m/m%]	15		
	Total fat [m/m%]	9,68	from which ω-3, ω-6	6,39
	Dietary fiber [m/m%]	8,75		
	Carbohydrates [m/m%]	4,2		
Minerals [m/m%]	Calcium	0,09	Potassium	0,263
	Magnesium	0,0785	Sodium	0,015
	Phosphorus	0,19		
Vitamins [m/m%]	Vitamin C	0,014	Vitamin B3	0,00051
	Vitamin E	0,0032		

This unique raw material offers a lot of opportunities for the development of healthy food products. It can be an excellent material for special diets, for example diabetic, reduced cholesterol, gluten-free, fat-reduced or vegetarian diets. The YASO can be widely used in food industry, in households, in public and in hospital catering (YASO, 2013).

3. CONCLUSIONS

If we use sprouted beans for raw material, the versatility of the products made from soybean is greatly increased. Additionally germination is a cheap, effective, mild and environmental friendly technology to increase the nutritional value of the soybean. So with this totally natural process we produced a product which is easy to use and has high nutritional value and quality. It has a neutral taste, smell and colour and has a proper consistency against to the other soy based products. YASO is internationally accepted and won two well-known awards in food fairs in the last year.

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BACTERIAL COMMUNICATION AND ITS IMPORTANCE IN FOOD MICROBIOLOGY

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SUMMARY
In contrast with past considerations, bacteria are highly versatile beings. They are able to communicate not only within their community but interspecies, and eventually to higher plants and animals. They do this producing specific signalling molecules inducing various gene expressions in other beings. This chemical communication is called quorum sensing. The lecture summarizes some recent information on this „social" behaviour of bacteria, particularly on its role in biofilms, food spoilage and food-related pathogenesis. Recent findings show that medically important antibiotics evolved in the nature not only to kill competitors but for communication. It is important to develop methods for detecting the signalling molecules and to understand better the quorum sensing inhibitors to develop new tools for enhancing food preservation and food safety.

1. INTRODUCTION

The Dutch *Anton van Leeuwenhoek* was the first observer of the tiny bacteria nearly three hundred and forty years ago. It is known today that bacteria are not only the most ancient but also the most abundant beings on the Earth. These single-celled organisms are also the major actors in food microbiology. The French *Louis Pasteur* and the German *Robert Koch*, great scientists of the second half of the XIXth century have laid the foundation of microbiology by making essential discoveries and developing fundamental methods resulting in the scientific explanation of food spoilage and many diseases (establishing the so-called „germ-theory"). Due to the far-reaching effects of their research, bacteria were regarded for long time as self-sufficient individuals making strictly unicellular life style and creating a single cause – single effect situation. This paradigm was accepted both in the food and medical sciences. However, mainly because the consequence of developments in the disciplines of microbial ecology and molecular biology during the second half of the XXth century, this type of former scientific approach is changing in the direction of studies for more deeper understanding of microbial communities. These developments resulted in the recognition, how sophisticated are apparently simple bacteria. It turned out that they are not simply existing together in various symbiotic forms with other beings, but they are communicating between each other as well as with other species, or, even with higher plants and animals. This communication means in its most well-known form the production and emission of specific organic molecules („autoinducers"), and induction of changes in behaviour of their own population or the other „partners".

2. QUORUM SENSING

This „social" interaction of bacteria is called „quorum sensing". By means of quorum sensing, bacteria perceive the cell density of their population. Quorum sensing affects also the spatial organization of the bacterial community, including phenotypical heterogeneity. It seems that the development of the quorum sensing phenomenon was a sort of early phase of the evolution of the multicellular organisms.

The release of signal molecules is proportional with the growth of the bacterial population and the detection of a minimal threshold stimulatory concentration of such autoinducer leads to the alteration in gene expression (resulting e.g. induction of transformation the growth phase into stationary phase, increased resistance or virulence of the

cells, sporulation, etc.). For interspecies communication, Gram negative bacteria produce acylated homoserin lactones (derivatives of certain fatty acids) while Gram positive bacteria produce certain oligo-peptides as autoinducers. Many Gram negative and Gram positive bacteria, living in mixed-species communities such as biofilms (see below) are using a quorum sensing system for cross-specific signalling. Certain polypeptides made by Gram positive bacteria are autoinducers for the producing organism but they inhibit other organisms.

There are intensive studies for getting be acquainted with the synthese of signal molecules, their recognitions by the target organisms, and for understanding the mechanisms of the relevant regulatory processes. The signal molecules diffuse easily through the cell membranes and interact with specific parts of DNA, regulating thereby RNA- and protein production. Quorum sensing circuits are involved in the coordination of certain virulence factors of bacteria causing plant- or animal-diseases. Such genes play an important role also in the development of biofilms.

3. THE ROLE OF QUORUM SENSING IN BIOFILMS

Bacterial biofilms are complex structures which can exist on natural surfaces as well as all types of artificial wet surfaces in processing plants. Actually, surface attachment is more common form of existence of bacteria than the free-living („planktonic") state. The importance of biofilms is evident for the food processing areas and the water treatment industry.

Bacterial communication plays a role in all phase of the biofilm formation. Quorum sensing processes „regulates" the cell densities of the bacterial populations, their metabolic activities and adaptation to nutrient sources. Bacterial cells forming biofilm and embedded in its exopolymer matrix have significantly different „transcription programs" than those of planktonic cells. Signal molecules regulates the release of cells into planktonic state from the „over-populated" biofilm community. Better understanding of all these processes are very promising both from the theoretical and practical point of views.

4. ANTIBIOTICS AS SIGNAL MOLECULES

Recent research shows that the interpretation of the role of antibiotics produced by certain soil bacteria should be modified: when they are used at unnaturally high concentration these microbially produced antibiotics can damage or kill other microbes, however, normally „they are talking, not fighting". At low concentrations, these compounds elicit self-protective biofilm formation or co-operative biofilm formation of mixed species. Eventually, they induce disposal of other microbes by causing them to increase their motility and „flee". Consequently, competition for resources by the microbe that produces the antibiotic is reduced.

5. COMMUNICATION BETWEEN THE GUT MICROBIOTA AND THE HOST INTESTINAL TISSUE

Although the communication between the symbiotic commensal bacteria of the gut microbiota and the intestinal tissue of the mammalian host organisms is a somewhat different area from our present subject, it should be mentioned here, because it is also a very important new field of research and host-bacterial crosstalk. Virulent microorganisms such as pathogenic bacteria are recognized by pattern recognition receptors and induce inflammatory responses in mammalian intestine. It became known in the recent decades that the gut

microbiota of the human organism contains more than 1000 different commensal bacterial species and they outnumber human cells by a factor of 10 to 1 without inducing inflammatory responses. The commensal bacteria-derived molecules mediate interactions between them and the host through sensing systems that may be different from those used for pathogenic bacteria. The commensal bacteria and the probiotic bacteria, recommended for consumption together with certain foods, exhibit beneficial functions in the host intestines. New findings indicate that the beneficial bacteria help to maintain the homeostasis of the intestinal environment.

6. ANALYSIS OF THE QUORUM SENSING SIGNAL MOLECULES

It follows from what has been mentioned before that development and application of methods suitable for detection of the signal molecules are important analytical tasks. The broad arsenal of modern instrumental analytical tools can assist this work. Developing relevant biosensors seems to be particularly promising, considering the rapidity, relative simplicity and economy of such measurements. It is hoped that by detection of the quorum sensing molecules the early detection of food spoilage may become possible.

7. INHIBITORY ASPECTS OF QUORUM SENSING

The search for quorum sensing inhibitors is also an interesting field of research. Quorum sensing inhibitors, if they are non-toxic for the consumers, would be especially important for food preservation. Dietary phytochemicals from plants known to have several health benefits and their relation to inhibition of bacterial communication is an important consideration in this regard. Inhibition of quorum sensing might be useful also in those technological operations where e.g. biofilm formation plugs filter membranes. As an example, it was possible to prevent biofilm formation by using an acylase enzyme. This inhibition based on the deactivation of the signal molecules mentioned in relation of quorum sensing of Gram negatives, increasing thereby the longevity period for effective use of such membranes.

8. CONCLUSIONS

Because quorum sensing plays important roles in food spoilage, bacterial biofilms and pathogenesis of food-borne pathogenic bacteria, bacterial communication in food ecosystems is a promising field of food microbiology. If bacterial communities and effects of their inhibition will be better understood, that can help the improvement of safety of the food supply chain, prevention of undesirable bacteria and development of methods for improved food preservation and food safety.

Since certain components of food inhibit, or, even support the formation of quorum sensing systems, and the bacterial communication in such ecosystems depends much on the microstructure and interactions between biotic and abiotic factors, better understanding of the relevant interactions could assist better prediction of shelf life of food.

9. RECOMMENDED READINGS

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COMPARATIVE STABILITY STUDIES AND POSSIBLE TRANSFORMATION ROUTES OF VARIOUS PHYTOSTEROLS

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SUMMARY

Phytosterols are natural plant sterols, these compounds are proved to decrease serum total and low density lipoproteins (LDL) – cholesterol level. The beneficial properties can be utilized either as enriched food ingredients in functional foods or as natural components of regular diet. The aim of the study was to develop novel phytosterol extraction and proper GC-FID methods and to examine the stability of phytosterols. During the analysis six standard compounds have been used (cholesterol, campesterol, brassicasterol, stigmasterol, β-sitosterol, stigmastanol) in twelve different plant seed oil samples (wheat germ, corn germ, line, sesame, poppy, pumpkin, soy bean, rape, hemp, dill, grape, walnut). Further aims were to study the stability of the sterol components for a long term storage period (one year) and to monitor the changes in the phytosterol concentration.

1 INTRODUCTION

Plant sterols are minor constituents of vegetable oils (Roche, 2010; Harrabi, 2008). Phytosterols belong to the triterpene family; they are not soluble in water. They occur in different forms, such as the free, glycosides esters form, or they can be esterified to fatty acids (Fernandes, 2007; Lagarda, 2006). Together with phospholipids and other glycolipids, phytosterols and their derivatives are essential components of plant biomembranes (Breinhölder, 2002).Their main sources are in unsaponifiable fractions of seeds and nuts (Alonso, 1997). The structure of these compounds are similar to that of cholesterol, therefore they can absorb in the human intestine instead of cholesterol (Roche, 2007), For this reason functional food products enriched with plant sterols have been developed. (Fernandes, 2007; Lagarda, 2006). The major plant sterol components are β-sitosterol, campesterol, stigmasterol, brassicasterol (CODEX STAN, 1997).

The aim of the study was the elaboration of a novel phytosterol extraction method, and the examination of phytosterol stability. Eight different extraction procedures were developed by adding additional purification steps and utilizing various concentrations of the saponifying agents. Cold pressed wheat germ oil with high level phytosterol content are suggested for food applications (Winkler-Moser, 2011; Normén, 2007). According to our own results the highest phytosterol concentration was also found in this product. For these reasons this sample was used during over examinations. The stability of six common dietary sterol compounds (brassicasterol, campesterol, stigmasterol, β-sitosterol, stigmastanol) were analysed for a period of one year in 13 different cold pressed vegetable oils.

2. MATERIALS AND METHODS

2.1. Chemicals

Phytosterol GC-standards (campesterol, β-sitosterol, stigmasterol, stigmastanol, bassicasterol) were obtained from Sigma Aldrich (St. Louis, USA) and the reagents (chloroform, potassium-hydroxide (KOH), methanol (MeOH), diethylether, n-hexane, 2,2,4-trimethylpentane, pyridine, hexamethyldisilazane (HMDS), trifluoracetic acid (TFA), sodium chloride (NaCl)) were obtained from VWR International (Radnor, Pennsylvania, USA).

2.2. Sample preparation

Sterol TMS ether derivatives were prepared based on the method reported Andrási at al. (2011). The concentration of the standard stock solution was 1 mg mL^{-1} chloroform. 125 µL Pyridine, 225 µL HMDS, 25 µL TFA have been added to the stock solution and this mixture was thermostated for 90 minutes at 70 ºC.

Eight methods have been studied for the preparation of the oil unsaponifiable fraction, methods 1-7 are shown on Figure 1. The first step was the same in all applied methods: one mL of 5-α-cholestane (internal standard) in tert-butyl methyl ether was added to 100 mg of wheat germ oil. METHOD 8: 100 mg oil samples were added directly the pyridine, HMDS and TFA.

After the saponification reactions extraction took place and the extracted derivatives have been separated from the organic phase and the solvent was later evaporated. The TMS ether derivatives were made using the above mentioned method. The residual materials have been redissolved in 2 mL 2,2,2-trimethyl-penatne.

Based on the efficiency comparison of the eight different methods, METHOD 4 has been selected for stability examinations. 13 different vegetable oil samples have been obtained and analyzed in one year long period (between June 2012 and June 2013). The oil samples have been stored at room temperature (in a dark storage .room).

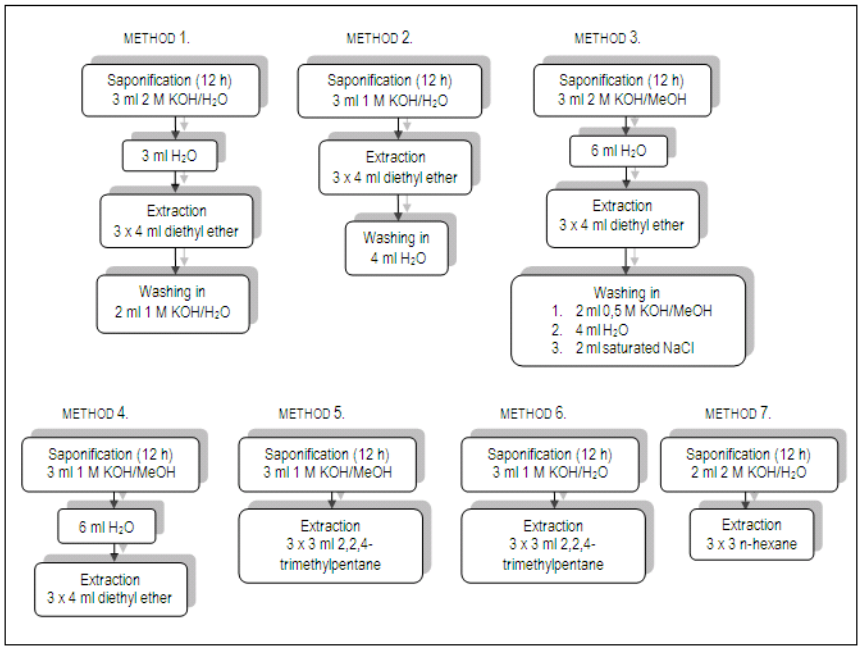

Figure 1: phytosterol extracted methods.

Sources: METHOD 1 (Cercaci, 2007; Rezig, 2012; Abidi, 2001); METHOD 2 (Cercaci, 2007; Rezig, 2012; Abidi, 2001); METHOD 3 (H. Kallel, 2008; Harrabi, 2008; Alonso, 1997; Pérez-Castaño, 2013; Li, 2007); METHOD 4 (Roche, 2010; Li, 2007, Abidi, 2001); METHOD 5 (Roche, 2010; Li, 2007, Abidi, 2001); METHOD 7 (Dutta, 1998; Normén, 2002; Amar, 2007; Alonso, 1997).

2.3. GC-FID determination of phytosterols

Analysis of the phytosterol TMS ether derivatives was performed using a HP-5ms capillary column (30m × 0.25mm, 0.25µm film thickness; Agilent Technologies, Inc., Loveland, Colorado, USA) in a Shimadzu GC-2010 gas chromatograph equipped with an FID flame ionisation detector. Samples (1µl) were injected using an AOC 20i autosampler and a split-splitless injector with a split ratio of 1:100. The oven temperature started at 100 ºC and it

was increased to 310 ºC at a 20 ºC/min rate. The injector and the detector temperatures where 250 ºC and 320 ºC, respectively. Helium was used as carrier gas at inlet pressure of 114.2 kPa, giving a column flow of 1 ml/min. Data were collected by Shimadzu GCsolution (version 2.30.00 SU7) software.

All GC experiments on phytosterol derivatized samples have been run in triplicate. The reference solution contained all steroid standard derivatives; the phytosterols content of oil samples was evaluated from the GC-FID chromatogram area of the standard solution.

3. RESULTS

Chromatographic comparison of the eight different extraction methods of wheat germ oil presented in Table 1. The sterol concentrations are given in mg/100 g oil. Recovery was calculated to 5-α-cholestane units. METHOD 4 was the most efficient method, the total phytosterol content was 1680 mg/100 g oil, and recovery was 74 %. In this case 3 mL of potassium-hydroxide (1M) was added to the oil samples for the saponification, after the addition of 6 mL distilled water, the samples were extracted three times using 4 ml of tert-butyl methyl ether. (Cercaci, 2007, Rezig, 2012, Abidi, 2001) The lower result has the METHOD 7. The cleaning steps, like washing with distilled water and KOH/MeOH (METH. 1-3), ceased the turbidity of the solutions but yielded lower recoveries. Saponification in water was applied, because unlike methanol, water doesn't mix with the solvents used for extractions and the separations are easier to perform. KOH/H_2O proved to be less efficient than KOH/MeOH, because fatty acids dissolve better in MeOH. In METH. 5, 2,2,4-trimethylpentane showed similar efficiency than diethyl ether. Saponification with KOH/H_2O yielded lower recovery even with the usage of 2,2,4-trimethylpentane. METH. 7 showed the lowest recoveries. The reason for this could also be that saponification with KOH/H_2O is less effective. METH. 8 is to represent the results without any saponification, using only derivatization. Compared to the other two solvents, extraction with diethyl ether was preferred because of the easier evaporation, even without using vacuum too. Method 6 showed the lowest overall recovery. The higher recovery has the METH 8, probably because by this method lack the extraction step.

Table 1: phytosterol TMS-ether derivatives of wheat germ oil.

phytosterol-TMS-ethers derivatives	METHODS (mg/100 g oil)							
	1	2	3	4	5	6	7	8
brassicasterol	1,1	1,9	1,7	2,0	2,0	10,4	n.d.	1,6
campesterol	71,4	74,4	149	204	142	19,1	16,4	37,9
stigmasterol	13,9	12,0	10,0	14,7	10,2	2,7	4,0	6,7
β-sitosterol	485	629	989	1337	1065	199	153	330
stigmastanol	34,0	30,1	95,7	123	68,5	2,4	n.d.	11,6
Σ	605	747	1245	1680	1288	224	173	388
Recovery (%)	81	69	67	74	67	30	70	90.
Corrected values by the recovery	722	977	1659	2122	1709	381	226	426

Results of the stability research met the expected requirements, so that the whole phytosterol content of oils didn't change significantly, that thanks to stabile steroid structure.

4. DISCUSSION

Eight phytosterol extraction methods were elaborated and compared. The phytosterol profiles were determined and long term stability of twelve oils were examined. Thermal stability has been monitored in progress, and further aims to identify the transformation products and purpose plausible pathways for transformation routes.

Table 2: long term (one year) stability of phytosterol-TMS-ethers derivatives.

oil samples	sampling date	mg/100 g oil						
		cholesterol	brassicasterol	campesterol	stigmasterol	β-sitosterol	stigmastanol	**Σ**
poppy	June 2012	n.d.	0,60	23,0	5,77	194	n.d.	**223**
	June 2013	n.d.	n.d.	20,3	5,33	183	n.d.	**208**
corn germ	June 2012	n.d.	0,26	71,9	43,3	462	78,5	**656**
	June 2013	n.d.	n.d.	60,9	35,9	432	29,8	**558**
walnut	June 2012	n.d.	n.d.	6,22	2,79	180	n.d.	**189**
	June 2013	n.d.	n.d.	5,48	2,38	142	n.d.	**151**
pumpkin seed	June 2012	n.d.	n.d.	2,80	3,27	166	24,1	**196**
	June 2013	n.d.	n.d.	3,73	5,08	151	29,9	**190**
hemp seed	June 2012	n.d.	n.d.	41,6	8,60	343	n.d.	**394**
	June 2013	n.d.	n.d.	32,0	5,42	302	n.d.	**340**
dill seed	June 2012	32,8	n.d.	n.d.	29,5	195	n.d.	**258**
	June 2013	24,5	n.d.	n.d.	29.9	175	n.d.	**229**
sesame	June 2012	n.d.	n.d.	70,9	30,1	471	n.d.	**572**
	June 2013	n.d.	n.d.	66,5	28,6	430	n.d.	**525**
grape seed	June 2012	n.d.	n.d.	14,4	17,3	121	10,5	**163**
	June 2013	n.d.	n.d.	13,7	16,1	119	9,01	**158**
linseed	June 2012	n.d.	9,66	45,1	10,7	227	n.d.	**293**
	June 2013	n.d.	5,11	37,9	20,4	163	n.d.	**226**
rape seed	June 2012	n.d.	56,5	148	1,86	424	35,4	**666**
	June 2013	n.d.	42,4	127	7,83	358	35,2	**570**
wheat germ	June 2012	n.d.	1,99	204	14,7	1337	123	**1680**
	June 2013	n.d.	7,11	118	21,2	561	121	**1542**
apricot seed	June 2013	n.d.	0,41	12,1	5,97	320	29,8	**369**
milk thistle	June 2013	62,2	4,04	17,6	28,4	155	33,2	**301**

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GENETIC AND MORPHOLOGIC CHARACTERISATION OF NOBLE ROTTED *BOTRYTIS CINEREA* ISOLATES FROM EGER WINE-GROWING REGION

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SUMMARY

*Botrytis cinerea has a very special role in plant protection and in oenology as well and characterisation of its features is very important in both points of view. In the course of this experiment, phenotypic and genotypic properties of Botrytis cinerea isolates were analysed from two small vineyards of Eger wine-growing region. 176 noble rotted isolates were collected during the year 2011 and were prepared by single-spore isolation. The isolates were identified by their common morphologic properties. The sclerotium production as a phenotypic, and variability of marker sequences (*β-tubulin gene, MSB1 minisatellite) *as a genotypic feature were analysed. In contrast of high morphological variability, a low genetic variability was noticed, with a few allele number and a low mutation rate in sequences. One special MSB1 minisatellite variant was identified which had been found earlier just in the Tokaj wine-region. The genetic differentiation analysis presented differences between the collecting times, therefore the population which caused noble rotting was not stable during the season.*

1. INTRODUCTION

Botrytis cinerea (teleomorph: *Botrytinea fuckeliana*) is a cosmopolitan haploid ascomycetous and heterothallic fungus that causes grey mould on grapevine and many other important crops in the temperate zone worldwide by infecting various tissues. In grapevine, the massive occurrence of *B. cinerea* prior harvesting will result in serious loss of fruit and a deterioration of wine quality. Development of grey mould (*pourriture grise*) on the grape berries is among the most striking viticultural phenomena with complex preconditions and implications (Vivier et Pretorius, 2002).

Botrytis infection requires moist conditions and if the weather stays wet, the malevolent form, "grey rot" can destroy crops of grapes. Grapes typically become infected with botrytis when they ripen, but when they are exposed to drier conditions, they become partially dried and the form of infection is brought about by the partial drying process which is known as "noble rot". When grapes are picked at a certain point during infection, they can produce particularly fine and concentrated sweet wine. The clue to these strategies lies in the understanding of genetic structure and dynamics of its populations.

Information about the populations and knowledge on the size and structure of *B. cinerea* populations is essential for the effective and economic protection against grey mould as neither the resistance genes of the host plant, nor the fungicides can maintain their effectiveness for an elongated period of time. Moreover, it would be highly advantageous both from the academic and the economic point of view to know if there is a difference between *B. cinerea* populations causing grey mould and noble rot. A plant pathogenic fungal population with a high level of genetic variation is likely to adapt more rapidly to fungicides or resistant host plants than populations with little or no genetic variations, and information on the level of migration between populations and on the presence or absence of sexual reproduction within a population may indicate how rapidly will novel (fungicid resistant or more pathogenic) genotypes spread between populations (Fournier et Giraud, 2008). Application of the tools provided by recent advances in molecular population genetics and biology is crucial in gathering that information.

The main characteristic of this fungus is its being the causal agent of the noble rot (*pourriture noble*), an infection that results in highly prized, sweet, smooth, full-bodied, special quality wines with a most pleasant bouquet. In the case of good climatic and ripening

conditions mainly in Tokaj, but in several other wine regions too, there is ~~a~~ potential for the production of noble rotted berries and wines. The unique and natural noble rotting process is very rare all around the world and because of this and diseases management, the economic importance of *B. cinerea* has inspired extensive research activity into its genetics, physiology, ecology, and epidemiology, as well as in the oenology (Elmer et Michailides, 2004).

2. MATERIALS AND METHODS

176 *B. cinerea* strains were collected from two small vineyards of the Eger wine district during the vintage period (September-November) in 2011 from two dedicated grape varieties Turán and Olaszrizling. Single-spore isolates were prepared from each isolation, and maintained on potato dextrose agar (PDA). The isolates were identified by their common morphologic properties. Morphological characterization was done after a 21-day 20 ^{0}C dark incubation on potato dextrose agar (PDA) by monitoring of the sporulation, mycelium and sclerotium production (Martinez et al, 2003). 96 isolates were chosen for genetic characterization, which was done by analyzing the β-tubulin gene and the MSB1 minisatellite sequence. Total genomic DNA was extracted from aerial mycelium of *B. cinerea* strains grown on PDA. MagnaLyser (Roche Applied Science) was used for the disruption of fungal cells, and DNA was isolated with the DNEasy Plant Mini Kit (Qiagen). The composition of PCR reaction was supported by automatic technique. Two unique sequences were amplified, the MSB1 and the *tub1*. A portion of *tub1* (Furnier et al, 2005) was amplified and sequenced using primers 155 and 1174, and the amplification of the intron of the ATPase gene (*atp1*) containing the microsatellite MSB1 (Giraud et al, 1998) was carried out by using primers MSB1fw and MSB1rev. The fragment analysis was performed in an automated single-capillary genetic analyzer (Qiagen). PCR products were purified and sequences were analyzed by LGC Genomics (Berlin, Germany). Editing and alignment of sequences were done manually by GeneDoc 2.6 (Nicholas et al., 2007), then the differentiation of genetic properties was investigated with DNASp 5.10.01 (Rozas et al., 2003).

3. RESULTS

3.1. Morphological variability

The sclerotium production of *B. cinerea* is the most common phenotypic feature (Elmer et Michailides, 2004). During morphological examination, the isolates presented a high sclerotium production and a low mycelium production ability. Most of the isolates produced a differently styled sclerotium pattern, therefore five groups were determined (S0, S1, S2, S3, S4, S5) to focus on sclerotium production ability and two groups (M1, M2) were separated to focus on mycelium production ability. No significant differences were found between the samples from the different grape varieties or the samples from the different vineyards. In the base of sequential collecting times, differences were found between the isolates, because the sclerotium production rate of isolates collected in late autumn (October, November) was higher than in isolates which were collected earlier (September) (Fig. 1). The high proportion of sclerotium production demonstrates the good adaptation ability of *B. cinerea* to the environmental effects in the base of late harvesting technology.

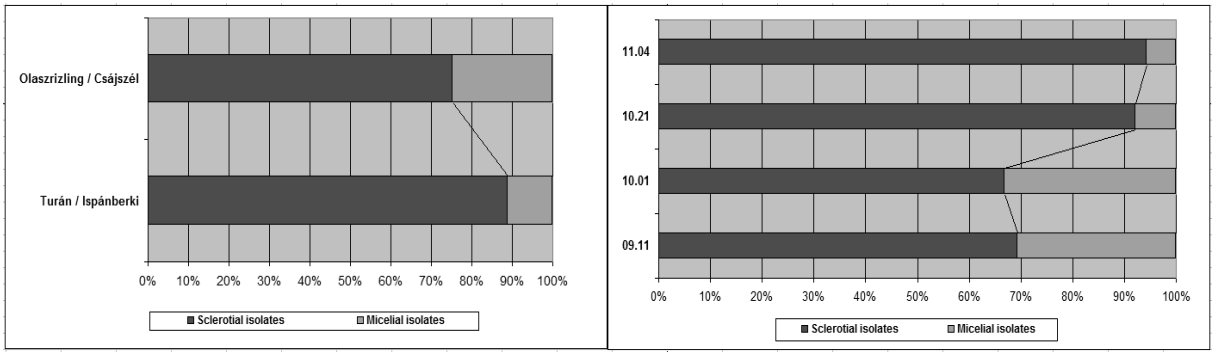

Figure 1: The percentage of mycelial and sclerotial isolates on the basis of grape vine cultivar and territory (left) and sample collecting time (right)

3.2. Genetic variability

In former studies high genetic variability was noticed in the case of genetically informative markers MSB1 and *tub1* in average and in samples from these territories (Váczy et al, 2007; Váczy et al, 2008). Contrary to earlier results, in case of MSB1 minisatellite a low allele number and variability was found. A moderate variability were found among the investigated sequences. The minisatellite allele map contrasts strikingly, the variability of alleles is great. However, there was one variant identified only in one isolate (b18H), which was also identified earlier but only in the Tokaj wine region, as well from one isolate. In connection with the β-tubulin gene, the population seemed to be homogenous, because just only one point mutation was found in this marker sequence.

3.3. Genetic differentiation

The investigation of genetic differentiation similarly to the morphological data, did not show differences between grape cultivars, but a clear difference was found between the populations of collecting times. Dissimilar genetic differentiation levels were found between the isolates collected in September and November, which could be indicative of the fact that the botrytis population that resulted in the noble rotting was not stable during the season (Table 1.). Comparing the data with former studies (Váczy et al, 2008) the differentiation between the sequences is significantly high, which shows the fluctuation of populations and confirms the idea of specification.

Table 1: The Gst values of MSB1 minisatellite sequences connected to sample collecting time, grape variety and vineyard. The Gst shows the genetic differentiation between the populations. The value is moderate between 0,05 - 0,15 high between 0,15 - 0,25 and very high over the value 0, 25.

SAMLPE COLLECTING TIMES	**1 October**	**21 October**	**4 November**
11 September	**0,066**	**0,123**	**0,127**
1 October		0,044	0,053
21 October			0,051
GRAPE VARIETY / VINEYARD	**Olaszrizling / Csájszél**		
Turán / Ispánberki	**0,017**		

4. DISCUSSION

In summary, the isolates showed a high morphological variability and a high sclerotial ability, but at the same time the genetic variability was low. The genetic differentiation analysis did not show differences between the isolates from the two grape varieties or from territories even so the sequential sample collecting times showed a changeable population structure in time. In the basis of phenotypical and genotypical results, presumably different botrytis populations took part in the noble rotting process of grapevine. Data underline a significant specialization of populations in this territory and these differences presumably are connected to that special and dissimilar "botrytis protective" grape protection technology, which was practiced here. The complexity and variability of this fungus make it difficult to manage and control, thus the high genetic variability and the sexual reproduction is an unambiguous answer for evolving rapidly and easily not just against fungicides, but for the changing of grapevine production in connection with late harvest time for producing noble rotted wines.

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DETECTION OF MILLING FRACTION OF CEREALS BY INFRARED SPECTROSCOPIC AND MICROSCOPIC METHODS

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SUMMARY
The aims of investigations were to elaborate macro- and microscopic infrared (IR) methods for testing and recognition of chemical components in plant materials as well as to develop spectroscopic and microscopic methods. Hungarian standard wheat milling fractions were produced under industrial conditions where samples were collected versus milling time. The main chemical components (such as starch, protein, lipid) and physical properties of fractions (particle size) were analysed by dispersive and Fourier transform (FT) spectrophotometers using visible (VIS) and near IR (NIR), and near and mid (MIR) regions of the electromagnetic radiation. Close correlation were obtained between the data of spectroscopic measurement techniques processed by various chemometric methods (e.g. principal component analysis [PCA], cluster analysis [CA]) and operation condition of milling technology. Based on the evaluation of the consecutive sampling spectroscopic data were pointed out, how uniform is the quality of given fractions in the technology versus time in other word how stable is the milling technology.

1. INTRODUCTIONS

The aspect of the nutrition the cereals are the most important vegetable food materials, because high in energy in the grain crops (containing 60-70% carbohydrate), they can be storage well, easy to process and have many uses. The average composition of the wheat grain is 71.9%, 12.2 %, 1.9 %, 1.7 % starch, protein, non-starchy carbohydrate and ash, respectively (Stanley, 2003; Lásztity, 1999). The milling fractions and the wheat test flour were examined by the classical, dispersive VIS/NIR spectrophotometer besides these the samples were measured by the more modern interferometric NIR/MIR spectrophotometer. In addition, microscopic examinations were made using NIR imaging technique. The aims were

- collecting data with parallel measurements by the VIS/NIR and FT-NIR/MIR spectrophotometers and hereby followed the milling operations;
- evaluating the spectra of analysed samples qualitatively by the help of multivariate data analyses (e.g. PCA, CA);
- starting initial optimization about the microscopic scans of the milling fractions.

2. MATERIALS AND METHODS

The wheat origin samples were produced by Gyermelyi Zrt. (Gyermely, Hungary) flour-mill. The reference materials originated from Sigma Chemical Co. (St Louis, MO, USA). There were gluten from wheat (G5004), unmodified wheat starch (S5127) and wheat germ oil (W1000). The main chemical components (such as starch, protein, lipid) and physical properties of fractions (particle size) were analysed by following spectrophotometers using three parallel measurements in case of each samples:

- FOSS NIRSystems 6500 dispersive VIS/NIR spectrophotometer with Rapid Content Analyser;
- Perkin-Elmer Spectrum 400 FT-NIR/MIR spectrophotometer with Near-infrared Reflectance Accessory and Universal ATR Sampling Accessory;
- Perkin-Elmer Spotlight 400 microscope.

2.1. Standard wheat milling fractions

Hungarian standard wheat milling fractions (BL55, BL80, BL112, TL50, where "BL" and "TL" mean "wheat flour" and "flour for pasta", respectively and the number after abbreviation refer to ash content in hundredth of %) were produced under industrial conditions where samples were collected versus milling time (at 8, 12, 16, 20, 24 and 4 o'clock of given days) and a special fraction called "wheat test meal" (BKL) were also taken and investigated 24 hours experiment. Another BKL sample set covered a sampling time of 150 hours following the mid-term stability of milling technology.

2.2. Closed system wheat milling fractions

Formerly referred to allied wheat, and more another 6 fractions were given us by the same factory, but these are meaning so-called closed system milling fractions (4th fracture, BL55, BL80, BL112, TL50 and BKL). Two kind of closed system milling process was realized: one of these included allied wheat, another was not (i.e. included mixture of different wheat species). On the first half of the day (at 7, 10, 13 o'clock) the aim was to get maximum yield of the TL ("flour for pasta") fraction, whereas on the second half of the day (at 15, 18, 21 o'clock) the aim was to get minimum yield of the TL. Of course not only the TL but also the 6 fractions were examined during each approach.

3. RESULTS AND DISCUSSION

The Fig. 1 is shown huge different between the TL50, BKL, 4th fracture and the BL fractions in case of the dispersive VIS/NIR spectra. BKL and TL50 spectra have a baseline shift between 400 and 700, and 1100 and 2500 nm, respectively by the standard wheat milling process. By the closed system wheat milling fractions the spectra of the 4th fracture differ from the BL fractions in the whole wavelength range. The BL fractions have to go through the sieve 100% undergoing at 315 microns and at least 95% undergoing at 250 microns during the sieve analyses, until the TL50 fraction 100% undergoing at 400 microns and at most 15% undergoing at 200 microns (Ministry of Rural Development, 2011). The effect of different particle size cause baseline shift in the range of PbS detector (1100-2500 nm), while the colour of the samples cause the main variability in the range of Si detector (400-1100 nm).

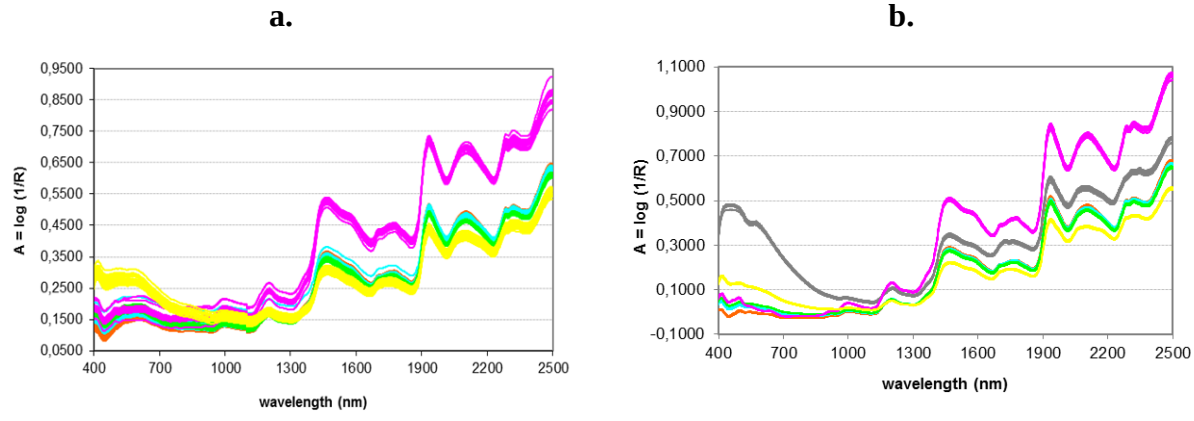

Figure 13: Dispersive VIS/NIR spectra of wheat fractions by standard (a) and closed (b) milling in the whole wavelength range (BL55, BL80, BL112, TL50, BKL, 4th fracture)

Using mathematical treatment (second derivatives) to reduce the baseline shift, which is caused by particle size differences and to highlight the more variable spectroscopic regions with the raw spectra in case of the dispersive and FT-NIR spectra by each process too. Comparing dispersive NIR and FT-NIR spectrophotometers according to the effect of the

second derivatives, FT spectrophotometer gives more detailed NIR absorption bands, but dispersive spectrophotometer gives more separable NIR spectra by the standard wheat milling process. Comparing samples with reference materials obtained that results the BL55, BL80 and BL112 increasing of lipids can be detected owing to higher ratio of bran and the aleuron-rich BKL and 4th fracture contain much more lipids (Fig. 2).

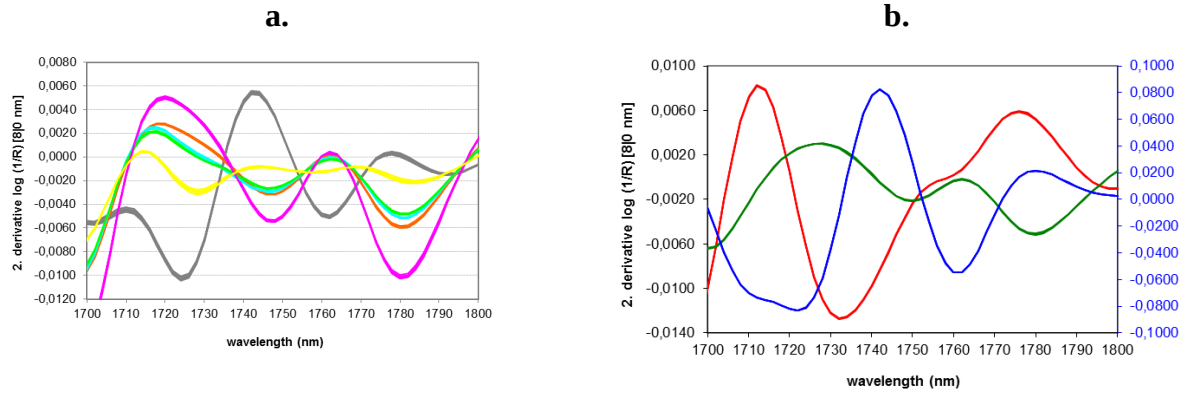

Figure 2: Dispersive NIR spectra of wheat fractions by closed milling (BL55, BL80, BL112, TL50, BKL, 4th fracture) (a) and reference material (gluten from wheat, unmodified wheat starch, wheat germ oil) (b)

If the spectroscopic data were evaluated by multivariate data analysis (PCA and CA), supplied more context between the different of the milling fractions at first, and aspect milling times secondly. Checking the five fractions by the standard milling process the scatter plots of score values of PCA and dendrograms of CA show distinct groups defined by products (Fig. 3). While the CA gives information just about the distance of the groups, until then from the PCA getting picture the direction of the groups too. In case of closed milling the six fractions are evaluated together. At first the 4th fracture, than BKL and finally TL50 separate from each other by the CA methods. The BL112, BL80 and BL55 fractions separate on the score of allied wheat or non by closed milling process.

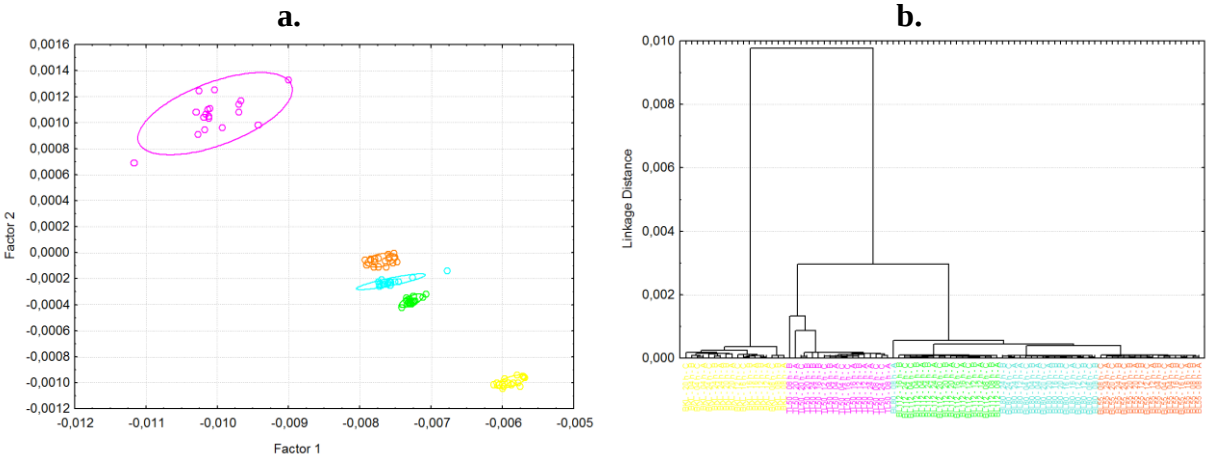

Figure 3: PC2 vs. PC1 score plot (a) and dendrogram (b) based on 2nd derivative dispersive VIS/NIR spectra (BL55, BL80, BL112, TL50, BKL) – the length of ellipses' horizontal and vertical projection onto the x- and y-axis, respectively are equal to the mean ± range

Besides the separation of the milling fractions, was another examination which applies to the milling time by the standard milling process. In the course of the 24 hours experiment PC2 vs. PC1 score plots show clear effect of milling time in case of BL112 and BKL fractions owing to its higher bran and aleurone content, respectively (Fig. 4a). The 150 hours experiment (which means 6 days monitoring, if checking the relative sampling times according to principal components) show that PC3 has sharp changes around 100 hours (Fig.

4b). So the time goes by the technology is not stable. By the closed milling process the samples were examined on the score of the maximum and minimum yield of TL with the CA and PCA methods, and there was not significant difference between the two kind of TL yields, but inside the BKL and TL50 fractions were sharper separation in some case.

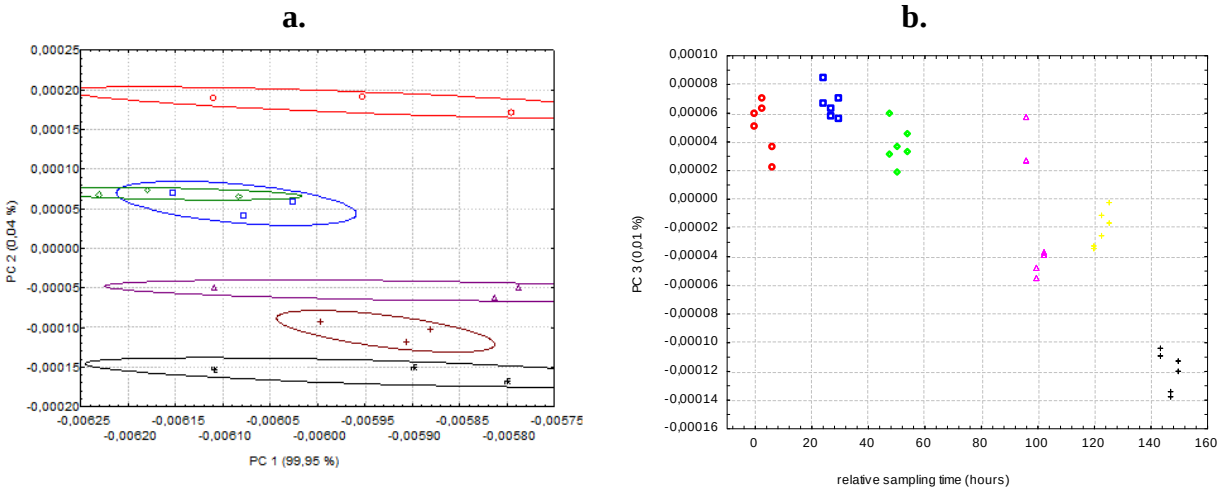

Figure 4: PC2 vs. PC1 (a) and PC3 vs. sampling time (b) score plots based on PCA of 2nd derivative dispersive VIS/NIR spectra of BKL fractions (a: 8, 12, 16, 20, 24, 4 o'clock; b: 1st, 2nd, 3rd, 4th, 5th, 6th sampling day)

The five fractions in case of the standard milling process was made a new attempt by the NIR microscope. Although this measurement with imaging technique keep going at present too, have obtained some promising results already. The spectra of FT-NIR images of different fractions were correlated pixel by pixel by the FT-NIR spectra of the wheat starch, the wheat gluten and wheat germ oil showing the distribution of the main constituents.

4. CONCLUSION

During the evaluation of the milling fractions was not just examined the difference between the fractions, then the quality of the fractions are how stable function of the time. Close correlation were obtained between the data of spectroscopic measurement techniques processed by various chemometric methods (e.g. PCA, CA) – such as physical and chemical fingerprints – and operation condition of milling technology. Based on the evaluation of the consecutive sampling spectroscopic dates it was pointed out, how uniform is the quality of given fractions in the technology versus time in other word how stable is the milling technology. Fractions with higher ash content (like BL112 and BKL) showed wider spectroscopic variation during milling time owing to variable bran content by standard milling fraction. Finally the results of the classical spectroscopic methods were followed parallel by NIR microscopy, which is still new technology in this research field.

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STABILITY STUDIES OF BIOLOGICALLY ACTIVE PEPTIDES IN REAL SAMPLES

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The stability of five biologically active peptides (Aspartame - APM, Alanyl-glutamine, L-Carnosine, L-Glutathione, γ-Glutamyl-Cysteine) has been examined under different environmental conditions in order to lay the foundation for food industrial applications. The effect of the pH, temperature (-18°C, +4°C, +30°C) oxidative environment, light stress (natural light irradiation) and the presence of different trace elements [Ca(II), Mg(II), Zn(II) and Fe(II)] has been investigated. Advanced liquid chromatographic and analytical (antioxidant activity with FRAP protocol) methods have been used to follow the concentration change of the target compounds in 15 or 30 days long time periods depending on the trace element content of the sample. The stability of the five studied peptides has been examined in starch solution (1%) as well in order to reveal effect of starch on lifetime of the given compounds. The applied matrix strongly affects the stability parameters of the given peptide. Neutral and basic pH facilitates the decomposition of Aspartame and L-Glutathione, similarly the temperature increase also accelerate the decomposition of the cysteine containing derivatives (L-Glutathion and γ-Glutamyl-Cystein). The strongest effect of the light stress was observed in case of L-glutathion and γ-Glutamyl-Cystein. The oxidative environment facilitated the decomposition of γ-Glutamyl-Cysteine and L-Glutatione significantly. Addition of transition metals to the peptide solutions led to enhanced stability in cases of all studied peptides, however differences have been observed depending on the metal/peptide molar ratio.

The peptide content of several food products (diary, raw and processed vegetable) has been compared with the aim of selection of the most effective sources of the studied bioactive peptides.

INVESTIGATION OF BIOACTIVE COMPONENTS OF BROCCOLI AS AFFECTED BY CULTIVATION CONDITIONS

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SUMMARY
Field experiments (irrigation treatments, foliar sulfur supplementation, spring and summer cultivation) were designed and performed in Gödöllő (2010-2012). The amount sulforaphane, total polyphenols, characteristic phenolic compounds (flavonoids and phenolic acids) were studied in broccoli as affected by cultivation conditions. The most important factor was the harvest date regarding the sulforaphan content and the phenolic components too. This tendency was the most characteristic in the year of 2011. Our data proved that cold climate (crop from November) is favourable for all investigated components. The sulfur supplementation seemed to be not so detrimental. The effect of irrigation resulted in different tendencies in all bioactive components in broccoli during the three years.

1. INTRODUCTION

Brassica vegetables belong to Cruciferous family possess both antioxidant and anticarcinogenic properties and provide a large group of glucosunolates. Aliphatic glucoraphanin (4-methylsulfinylbutyl glucosinolate) is the most predominant glucosinolate in broccoli and is hydrolysed by the enzyme myrosinase to sulforaphane when it is released from the plant vacuoles after mechanical stress, e.g. during cutting or chewing (Rochfort et al., 2006).Sulforaphane is the active component that inhibits Phase I enzymes and induces Phase II enzymes which is thought to result in certain cancer protection. Variation in the antioxidant contents of Brassica is caused by many facrors: variety, maturity at harvest, growing condition, soil and condition of post-harvest storage. Broccoli is also a source of flavonol and hydroxycinnamoyl derivatives (Podsedek, 2007). The aim of the study was to investigate the effect of cultivation circumstances (harvest date, sulphur supplementation, irrigation) on the amount of some bioactive components in broccoli florets.

2. MATERIALS AND METHODS

2.1. Plant materials

In the years of 2010, 2011 and 2012 all field experiments (irrigation treatments, foliar sulphur supplementation, spring and summer cultivation) were designed and performed in Szent István University, Gödöllő (Pék et al., 2012).

2.2. HPLC analysis

Sulforaphane: hydrolysis of glucoraphanin to sulforaphane with 0.1 M HCl and its extraction (twice with 25 ml of dichloromethane) was made according the method of Nakagawa, et al., 2006. Sulforaphane was determined by a modified RP-HPLC procedure of Liang et al., (2006) and it was separated with a NUCLEOSIL 100-C18, 3µm, 250 mm x 4.6 mm column and a gradient elution of A (distilled water) and B (acetonitrile) was applied.

Phenolic components were extracted with 2 % acetic acid in methanol. Chromatographic separation of phenols was performed on EC NUCLEODUR Sphinx RP, 3µm, 150 mm x 4,6 mm column using gradient elution of A (1 % formic acid) and B (acetonitrile).

For quantitive determination the compounds were detected at their absorption maxima (195 nm for sulforaphane; 280, 320 and 355 nm for phenolic components).

Peaks of sulforaphane and phenolic components were identified by comparing retention time and spectral properties with those of standard materials, which were used as external standard for the quantitative determination.

2.3. HPLC instrument

A Waters Alliance chromatograph consisting of a Model 2695 Separation Module and a Model 2996 photodiode-array detector was used. The chromatograph was operated by Empower software.

2.4. Measurement of total phenolics and antioxidant activity

The total polyphenol content of the samples was determined according to MSZ 9474-80 (1980) with Folin-Ciocalteu reagent and was given in GAE (mg/kg). For the evaluation of antioxidant activity DPPH free radical (Brand-Williams, et al., 1995) was added to the extracts (5 g sample+ 20 ml methanol, overnight stored at 4 °C and filtered) and was expressed in TEAC mmol/kg.

3. RESULTS

3.1. Sulforaphane

Considering the effect of the irrigation treatments, foliar sulphur supplementation, spring and summer cultivation on the sulforaphane content our results showed in the year of 2010 that the samples harvested later (at the end of October) contained 59-95 mg/kg sulforaphane, as it seen in Figure 1.

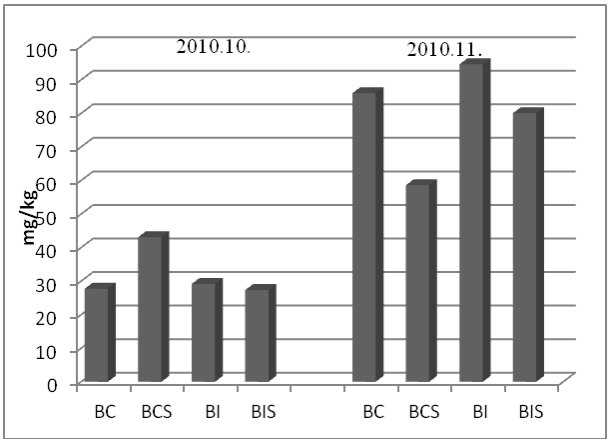

Figure 1.Sulforaphane content in 2010 in control(BC), sulphur supplemented (BCS), irrigated (BI) and irrigated and sulphur supplemented samples

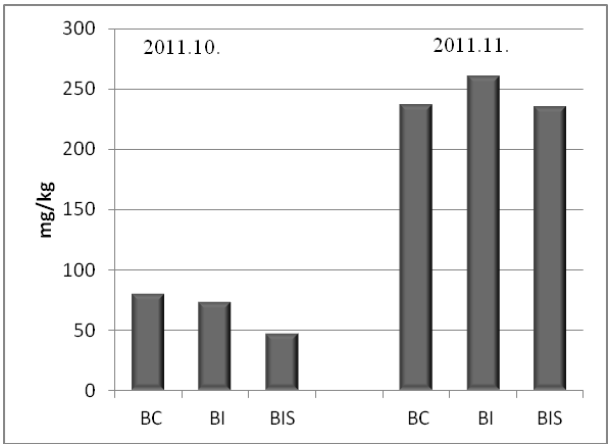

Figure 2: Sulforaphane content in 2011 as a function of irrigation (BI) and sulphur supplementation (BIS);n=3, RSD:10-20 %

The sulphur supplementation had no positive effect. In the next year (2011) the most important factor was again the cultivation way. The broccoli florets harvested in October and November contained 47-80 and 235-260 mg/kg sulforaphane respectively (Figure 2). In the third year (2012) sulforaphane was determined in the range of 32-43 mg/kg in the samples irrigation had no effect on the sulforaphane content.

3.2. Phenolic components

10-12 members of phenolic compounds of broccoli florets (flavonoids and phenolic acids) were identified in the study. In the year of 2010 the irrigation affected differently in the samples harvested in the beginning of October; the amount of ferulic and sinapic acid polymers decreased with 20-33 %. On the other hand the ferulic acid (BC:0,7 and BI:14,4 mg/kg) and chlorogenic acid (BC:0,7 and BI: 13,3 mg/kg) content increased significantly. The sulphur supplementation alone did not show great differences in the results generally, but the irrigation and sulphur supplementation were not favourable for the phenolic components.
In the year of 2011 the irrigation had no consequence effect to the samples collected in different cultivation period, but it was clear that the samples harvested in the middle of November contained the highest amount of the most characteristic phenolic components. The data showed that the most important factor was the harvesting date, the cool climate as it can be seen in Figure 3.

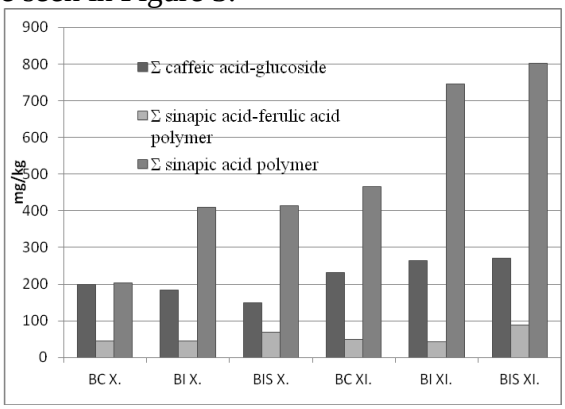

Figure 3: The effect of irrigation in 2011 on three main phenolic compounds BC-broccoli control; BI-broccoli irrigated; BIS-broccoli irrigated, sulphur supplementation, n=3, RSD:10-15 %

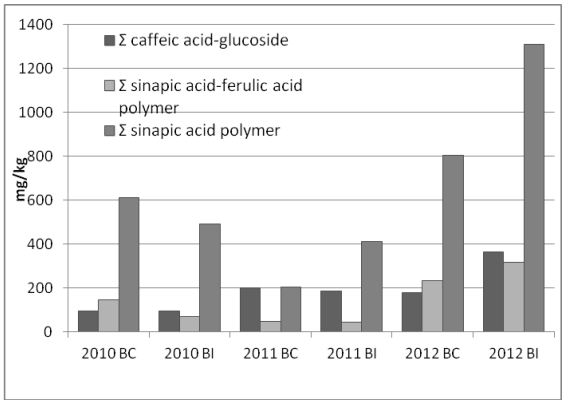

Figure 4: The most characteristic phenolic components as a function of irrigation. BC-control; BI-irrigated, n=3, RSD:10-15

In the third year (2012) the amount of all characteristic phenolic components was higher in irrigated samples harvested in October. The results are summarized in three years in the Figure 4.

3.3. Total polyphenols and antioxidant activity

In the year of 2010 the irrigation and sulphur supplementation did not influence in a great extend the amount of the total polyphenols. In the next year (2011) the samples harvested in November contained higher amount of polyphenols (895-989 mg GAE/kg) than those which were collected in October (733-812 mg GAE/kg). Otherwise the effect of irrigation and sulphur supplementation was not significant. These data proved that cold climate (crop from November) is favourable for the polyphenol content. In the third year (2012) polyphenol content was higher in the raw samples (harvested in October) without irrigation (control:726 mg, irrigated:684 mg GAE/kg) but the difference was not significant.

4. CONCLUSIONS

Considering the various treatments the most important factor was the harvest date (cultivation) regarding the sulforaphane content and the polyphenols as well. This tendency was the most characteristic in the year of 2011. The sulfur supplementation seemed not to be so convincing. Rangkadilok et al., (2004) studied the effect of sulfur fertilizer (three gypsum applications) on glucoraphanin levels in three broccoli cultivars at different growth stages.

They found that the sulphur (S) treatment during the early vegatative phase increased S uptake and the glucoraphanin content in each plant organ. The results of Ciska (2000) showed that the year x cultivation interaction modified the glucosinolate content of vegetables.

ACKNOWLEDGEMENT: *This research was performed with the support of the National Development Agency (project no.: TECH-09-A3-2009-0230).*

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IDENTIFICATION OF *LACTOBACILLUS* SPECIES BY MEANS OF MOLECULAR METHODS

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A significant proportion of fermented food products is made with the use of *Lactobacillus* species. Determination of microbiological composition of these foods is mainly performed by the classical microbiological techniques (selective media etc.). In the course of our experiments we had two main goals: 1. Collecting lactobacilli of food origin that might be used in food (mostly dairy products); 2. Testing and adapting molecular biology tools for rapid and exact determination of the isolated *Lactobacillus* species.

Several different food samples were investigated for presence of *Lactobacillus* species. For lactobacilli, MRS agar plates were used as selective medium. In the first step the species were identified by means of their phenotypic morphology. This experiment was followed by molecular investigations. DNA was extracted from each isolates, then the E8F-E1115R general bacterial primer pair was used to amplify an approximately 1100 bp long fragment of the 16S rDNA. These fragments were sequenced and generally the species could be identified; nevertheless, sequencing was not able to distinguish members of the '*Lb. casei*' group (*Lactobacillus casei, Lb. paracasei Lb. rhamnosus* and *Lb. zeae*). In order to resolve these species, the same 16S rDNA fragments were digested simultaneously by different restriction enzymes (RFLP). Standard restriction patterns were generated from the amplified 16S rDNA fragments of reference bacterial strains and the patterns were compared to those of the newly isolated strains. At the same time species-specific primers were used for separation of these species.

In our experiments we managed to identify *Lactobacillus* strains of different origin by means of molecular techniques. Though the RFLP method is widely used nowadays, we showed that combination of three enzymes is needed in order to distinguish members of the '*Lb. casei*' group. Sequencing was a very useful technique, as well. In case of dubious results the use of species-specific primer pairs is advised for the exact identification.

DEVELOPMENT OF A NEW METHOD BY USING NIR DEVICE FOR PROMPT CLASSIFICATION AND AUTHENTICATION OF MILK SAMPLES OF DIVERSE ORIGIN

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SUMMARY

Aim of the study is to develop a spectroscopic — thus, non-invasive and not reagent-demanding — method for recognition of origin (the animal species) of milk samples, particularly for investigation of mare milk which can be regarded as a Hungarian specialty in Europe. Further goal is to develop quantitative calibrations for the estimation of the m/m% ratios of milk mixtures, with regard to the origin, and for differentiation of them from some cheep artificial additives as whey powder or bovine milk powder etc.

1. INTRODUCTION AND PRELIMINARIES

Near infrared spectroscopy as a tool of qualification and detection of adulterants is not uniformly widespread in all areas of food industry. There are many publications about NIR investigations of adulteration of cereal products (M. Cocchi) These researches can be deduced to the early 90s. Another branch of food processing where researches of NIR based qualification and authentication are intensive, is the meat industry, since the late 90s-early 2000s. (Cozzolino) But in the dairy industry there are no intensive studies on the NIR or mid-IR methods and the potential uses of them as in the aforementioned segments of food trade. Three publications were found about attempts on recognition of origin of milk or milk product samples. One work deals with authentication of cheese samples. The aim of authors is to quantify ratios of different (cow, ewe, goat) milks in cheese samples (J. G.-Martín), working with a NIR fiber-optic probe immersed to the sample and perform a PLS data processing method on the spectra. Two studies were found about mid-IR spectroscopy based qualitative classification of milk samples with regard to their origin (N. Nicolau) (C.S. Pappas et al) In these works authors do not touch upon quantitative methods. Pappas et al. investigate the mid-IR spectra of defatted goat and sheep milk samples. They perform cluster analysis based on primarily protein bands and phosphorus-oxygen vibration signals; the differentiation of the two species is successful.

2. MATERIALS AND METHODS

2.1. Samples investigated:

Twenty six bovine, sixteen sheep, twenty six goat and twenty two equine individual milk samples and 5 artificials (milk and whey powders) were taken. Note that some ewe and mare milk samples showed characteristic intra-specific differences because they were originated from strongly different types of the investigated animal species. For example ewe samples were collected predominantly from Merino animals but we also collected a couple of Tsigai, Tsikta, Awasi etc. samples.

2.2. Sample preparation

Raw milk samples were lyophilised in a CHRIST 1-4 LCS apparatus. It was necessary to get rid of the water because the —OH signals of it suppress many other NIR bands which can be important in origin differentiation (see Figure 1).

2.3. Collection and handling of spectra

A Bruker MPA 650 Multi-purpose Analyser was applied in Integrating Sphere mode and Opus 5.5 software package. PCA for qualitative evaluation of spectra were performed with Windows SPSS 16.0 software. Quantitative calibrations were made with Opus 5.5 Opus Quant software. Samples were investigated in pairs.

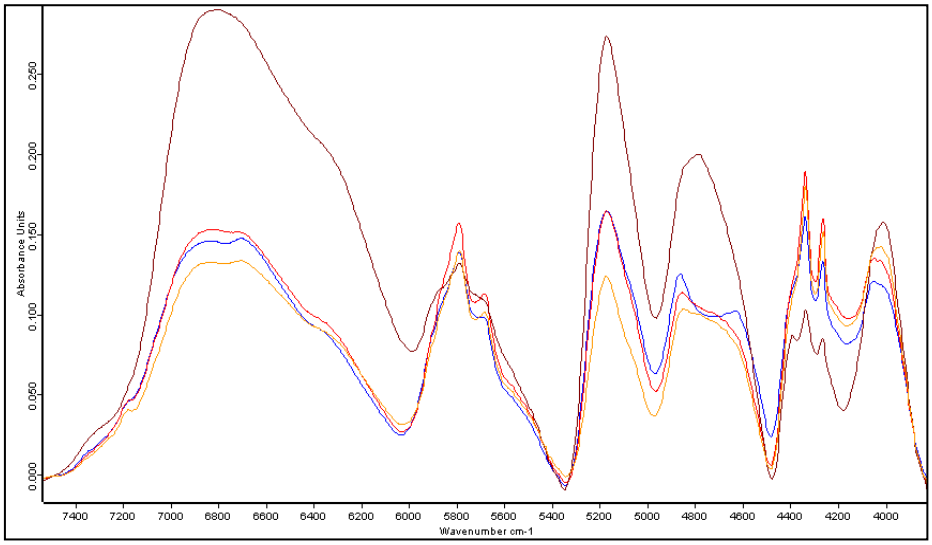


Figure 1: Spectra of freeze-dried bulk milk lyophylisates of each species, between 7500 and 3500 cm-1.

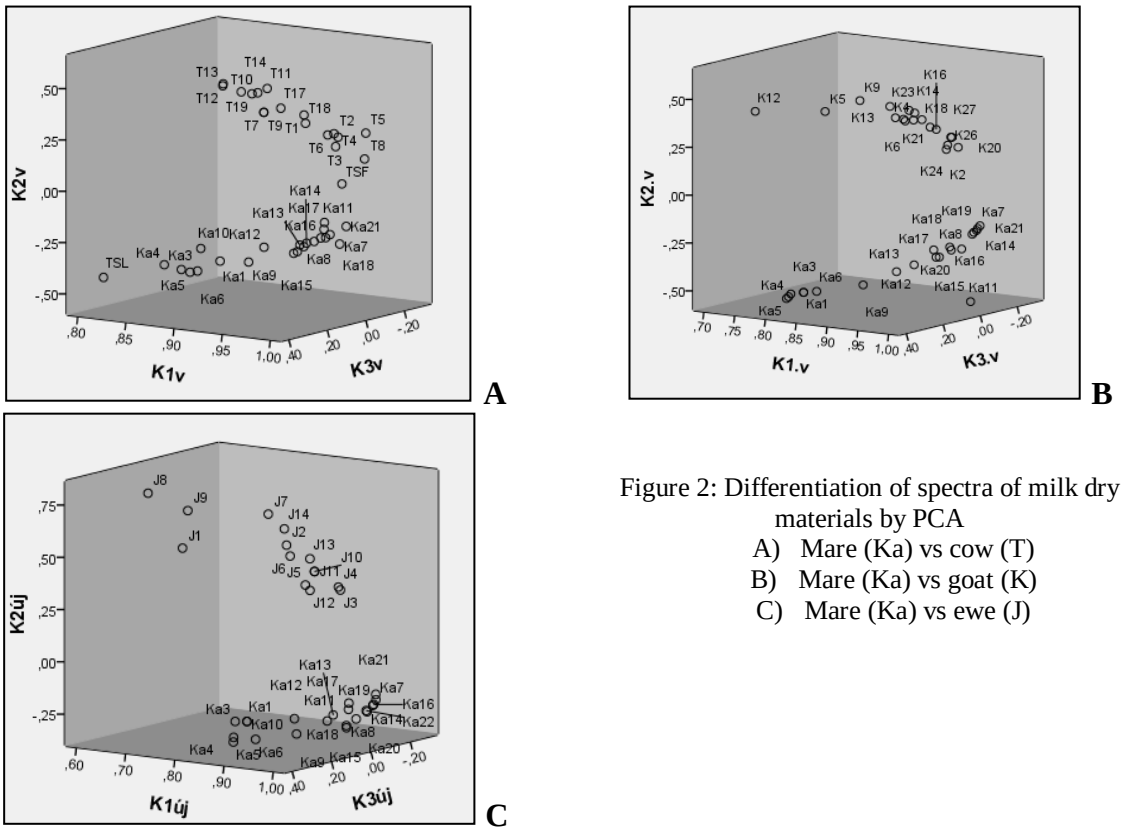
3. RESULTS

3.1. Some characteristic traits of the spectra of dry material from different milks

For equine milk, the main features were the extremely strong signals at 7500-6000 cm^{-1}, and 5200-5000 cm^{-1}. These were carbohydrate —OH bands. With regard to results of our complementary measurements we found that the equine milk contained 5-7 m/m% (raw weight) total carbohydrates while for the cow and goat samples it was 3,5-4,5 m/m% and for sheep the total carbohydrate was 3-5 m/m%. Other traits were the suppressed protein signals and the lipid regions at 4600-4200 cm^{-1} which were less intensive than in other species (it was expected because equine milk has poor fat contents). The characteristic of the sheep milk was the sharp doublet at 4850 and 4630 cm^{-1}, probably originating from two protein N—H—C=O signals Amide A and Amide II+III. (See plot Figure 1.) For bovine and caprine samples there were no as easily recognizable bands as the aforementioned ones. As a result, bovine/caprine differentiation was the least effective and quantification showed the largest error (see below).

3.2. Qualitative classification of the milk samples by PCA

The PCA investigation of the whole spectra did not give adequate distinction between species but if certain regions — including the carbohydrate signals in the intervals 7500-6000 and 5200-5000 cm^{-1} and a region of miscellaneous signals 4990-4470 cm^{-1}— were chosen, the classification became more successful. Note that while there were jutting points and serious inhomogenities the groups of the milk samples (as consequence of the intraspecific differences and of the different stages of lactation), no misclassified samples were found in the groups (see plots Figure 2). The species-dependent changes in intensity and signal shape in these regions were observed to be the base of the quantitative estimation of mixing ratio of milk dry materials. Those spectral intervals were chosen for calibrations, where PCA showed the sharpest differentiation between species.



A B C

Figure 2: Differentiation of spectra of milk dry materials by PCA
A) Mare (Ka) vs cow (T)
B) Mare (Ka) vs goat (K)
C) Mare (Ka) vs ewe (J)

3.3. Attempts to develop a method for quantitative distinction between different milks

Calibrations were made with bulk milk of the investigated species (milks containing more than one individuals of the same species) because in the industrial practice bulk/tank milk are in use many times. The investigated combinations were mare/ewe, mare/cow, mare/goat, goat/cow, ewe/cow, and ewe/goat. Each calibration covered the whole 0-100% interval of the possible mixing ratios. Validation were performed with i) ten mixtures of bulk milks ii) ten individual milks of the species which the calibration covers. For main data of calibrations see Table 1. It shows that the correlation was relatively good and in 4 cases out of 6 the robustness was also sufficient.

Table 1: Main statistical characteristics of the six quantitative calibration

Case	R^2, %*	RMSECV, m/m%	PLS factors (max. 10)	Robustness
Mare/ewe	99.11	2.75	5	optimal
Mare/cow	99.55	1.90	9	serious overfitting
Mare/goat	98.73	3.10	7	sufficient
Cow/goat	97.56	4.69	8	slight overfitting
Cow/ewe	99.74	1.47	7	sufficient
Goat/ewe	99.30	2.63	7	sufficient

*in the region 7506-3760 cm^{-1}

For validation with bulk milks sufficient results were obtained with 5 of the 6 calibrations. For these the error percentage of the prediction of the mixture ratio (m/m%) of a sample did not exceed 5% for at least 8 of the 10 validation mixtures. The exception was the cow/goat case where 3 samples out of 10 were mispredicted. An unexpected observation was that this two milk types showed the least spectral differences (which could cause the mispredictions) yet the calibration of their mixture ratios did not show overfitting.

Validation with individual milks was unsuccessful. The mixture ratios (which in this case were 0% or 100%) of these samples were seriously mispredicted. Thus, these calibrations can be used only for bulk/tank milks where the individual differences (e. g. of the lactation stage of the animals) are equalized.

4. CONCLUDING REMARKS

According to the results but with regard to the limited number of the investigated specimen it can be declared that the NIR based differentiation of equine, bovine and ewe milks can be performed from the spectra of freeze-dried samples. The signals in the spectra which bear the differences were probably carbohydrate and protein based bands in the 7506-3760 cm^{-1} region. Qualitative distinction of the milks (species) by PCA was successful. Investigations aiming quantification of the mixing ratio of milk lyophilisates of bulk milk were fruitful, except one case. But the build-up of properly robust quantitative calibrations requires further work. It is necessary to take further samples of different varieties, age and lactation period of each species into investigation to make clear the influence of these parameters to the spectra and thus to develop adequate quantitative methods.

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THE EZ-PRODUCT FAMILY

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Make your microbiology workflow EZ

The EZ-Product family includes the EZ-Fit™ Manifold, the EZ-Pak® Dispenser Curve, the EZ-Stream™ Pump and the EZ-Fluo™ Rapid Detection System. In combination, these products provide optimal performance and streamline your bioburden analysis workflow. The EZ-Fit™ Manifold makes laboratory filtration easier. Its unique design includes quick-fit connections for assembly and disassembly without tools and a low profile to increase operator comfort. Plus, all internal areas are accessible for easy cleaning, facilitating biofilm prevention. Different filtration heads, all with quick-fit connections, make the manifold compatible with disposable filtration devices, stainless steel and glass funnels. The second member of the product family is the EZ-Pak® Dispenser Curve. The dispenser provides high-speed sterile membrane dispensing with no-touch operation. The new, improved design allows for quick and easy membrane loading.Completing your filtration set-up, the EZ-Stream™ Pump increases efficiency. Fluid flows directly through the pump to waste, eliminating the need for intermediate waste containers. The pump is designed for quiet operation, and the vacuum level is compliant with regulatory standards. The EZ-Fluo™ Rapid Detection System is an easy-to-use, non-destructive, fluorescent staining-based system for rapid detection and quantification of microbial contamination in filterable samples.

EZ-Fit™ Manifold

The EZ-Fit™ Manifold was designed to minimize contamination risks while making laboratory filtration more convenient.

Reduce the risk of false results. When filtering water, beverages and other liquids, residual amounts of sample can become trapped in inaccessible areas of the filtration equipment. This can lead to biofilm formation, which can eventually cause false positive results by contaminating the test. The unique design of the EZ-Fit™ Manifold makes it easier to prevent biofilms. Each component can be removed without the need for tools. All internal areas are accessible and can therefore be cleaned easily. The shape and position of the 2-way valve prevent the user from accidentally touching the filtration head while opening or closing the valve. To avoid liquid back-flow from the vacuum system, a check valve is integrated into the flexible tubing connection.

Flexible setup. Not only does the tubing have a check valve, but it can be connected to either side of the manifold to fit your work setup. In addition, the EZ-Fit™ Manifold is compatible with a variety of filtration heads, all with quick-fit connections, accommodating both reusable and disposable filtration devices. The new filtration head for Microfil® was designed to improve ease-of-use, reduce maintenance and limit the risk of contamination. A continuous rim allows easy and safe membrane transfer with forceps and membrane removal with one hand. Another filtration head accepts standard N°8 rubber stoppers which fit Merck Millipore devices such as Monitor 55-Plus™, Microfil® V and Microfil® S. One filtration head is also compatible with stainless steel and glass funnels.

Ease of Use. The 2-way valves were designed for optimal ease of use. They are large and easy to operate. The manifold design also incorporates a low height to increase operator comfort, especially when used in laminar flow hoods. Setup is easier, too, with a unique system to ensure the stability even in non-flat working areas. Finally, the total weight is only 2.9 kg (for a 3-place manifold) so it's easy to move the manifold to where you need it.

EZ-Pak® Dispenser Curve

The EZ-Pak® Dispenser Curve makes membrane dispensing faster and easier.

Faster loading. The newly designed EZ-Pak® Dispenser Curve allows you to load a cartridge in less than 30 seconds. Each cartridge contains 150 membranes, and when the dispenser is loaded the membranes are automatically positioned for perfect dispensing.

Faster operation. An industrial infrared sensor permits no-touch dispensing of one membrane in less than a second. There is no longer a need to press a lever and risk contamination. The membrane is consistently well positioned for easy transfer with forceps, so the whole procedure can be done one-handed. Thank to its unique design, EZ-Pak® Dispenser Curve can be easily moved between lab benches and its smooth shape with facilitates cleaning.

Flexibility and quality. A wide variety of membranes covers any application, with pore sizes from 0,2µm to 0,8µm, diameters of 47mm or 50mm, and white, black or green membranes. The imprint of pore size and catalog-lot- and sequential number on each membrane cell facilitates traceability. Pore size, flow rate, extractables, retention, recovery and sterility of the membranes are all tested and listed on the Certificate of Quality.

Robust operation. All the components of the EZ-Pak® Dispenser Curve are selected for durability and high-throughput dispensing. With the sensor one and only one membrane is dispensed, every time. Furthermore, the dispenser can be used cable-free: when the Lithium ion battery is fully charged up to 10 000 membranes can be dispensed.

EZ-Stream™ Pump

Specially designed for microbiology analysis, the EZ-Stream™ vacuum pump is at home handling both liquids and gases making it suitable for many additional Life Science Laboratory applications.

Thanks to its compact design the pump is ideal for use on both the work bench and in the laminar flow hood where its small footprint (W 20cm – L 17cm – H 22cm) will save precious space. The vacuum is provided thanks to a maintenance-free diaphragm and specific check valves that allow liquids to run through the pump. Simply connect the pump to your manifold or filtration apparatus and place the discard tubing to drain and you are ready to filter.

There is no need to use any waste containers normally associated with traditional air pumps or an in-house vacuum. This brings an additional gain of space in the laboratory working area and eliminates the necessity for routine emptying of heavy waste containers.

Thanks to its innovative design, the EZ-Stream™ pump can provide vacuum up to 600-700 mbar, consistent with ISO 8199 standard on water quality. Direct liquid transfer through the pump head translates to high flow rate performance. The pump can easily handle a multiple head manifold for water or beverage testing, providing a comparable flow rate to traditional vacuum/pressure pumps and a better flow rate when compared to other liquid pumps. Furthermore, the EZ-Stream™ assembly has been optimized to reduce the noise and improve operator comfort. Aligned with GLP, decontamination of equipment is an efficient and easy operation thanks to the smooth design of the pump.

- **No handling of liquid waste**
- **For microbiological analysis, according to ISO standard**
- **Noise reduction design**
- **Compact design**
- **High performance flow rate**
- **Maintenance-free technology**

EZ-Fluo™ Rapid Detection System

A fast, non-destructive, fluorescent staining-based system for microbial detection

- **Rapid technology capable of providing results in 1/3 of the time required using traditional techniques**
- **Flexible approach based on standard membrane filtration and reduced incubation on media plate**
- **Non-destructive method, compatible with any ID technique**
- **Compact hardware, fits easily on any laboratory bench**
- **Cost effective solution for routine use as well as investigation**

Faster product release

Today, more and more QC laboratories are switching to rapid methods for the detection of microbial contamination. This is because traditional methods require several days to provide results and therefore are of limited value when it comes to releasing product to the market faster, or to conducting quick investigation when necessary.

EZ-Fluo is a non-destructive method that enables you to continue to grow the microorganisms after they have been stained in order to identify them using any standard ID technology. This is a tremendous advantage compared to other rapid test systems which cannot identify microorganisms using the same sample that was tested to detect the contamination.

Applications

EZ-Fluo can detect and quantify microbial contamination in any filterable sample. Potential applications are numerous and include testing of the following sample types:

- **Raw materials**
- **In-process samples**
- **Final product**
- **Environmental samples**

Simple protocol using regular membrane filtration and incubation

The EZ-Fluo Rapid Detection System consists of a reader and staining reagents used in combination with regular 47mm filtration membranes and standard media. Optimal results can be obtained using Merck Millipore membranes and comprehensive media portfolio. The use of the camera (optional) offers traceability of results and comfort in reading.

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VARIETY DEPENDENT ANTHOCYANIN PROFILES OF ELDERBERRY CULTIVARS (*SAMBUCUS NIGRA* L.)

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SUMMARY
Today, utilization of natural plant colorants is a preferred alternative to synthetic food colour agents to adjust the colour of foods. Natural colorants usually mean natural pigments that are extracted and concentrated from colourful plants. Colouring ability of elderberry, a widely used colouring food, is experienced to be highly cultivar dependent, however, it also depends on factors such as the geographical origin, climatic conditions and the date of harvest. In our research, anthocyanin profiles of one Austrian (Haschberg) and four Danish (Sampo, Samocco, Samyl, Samident) elderberry varieties were investigated during ripening with HPLC-VIS and HPLC-ESI-qTOFMS analytical techniques. The aim of this work is to reveal the compositional differences that are responsible for the varying colouring abilities.

1. INTRODUCTION

The study of natural colorants is an extensive and active area of investigation due to the growing interest of substituting synthetic colorants, since the latter has been recently associated with adverse effects in humans (Chou et al., 2007). Therefore, food producers try to replace their synthetic food coloring agents to natural food colorants, especially since the appearance of the 1333/2008/EC regulation. It states that foods containing artificial coloring material must be marked with the following phrase: 'may have an adverse effect on activity and attention in children'. Therefore natural food colours are more consumer-friendly and because their resource is usually of plant origin, consequently, they do not require declaration with an E-number. By nature, chemical forms and the amount of pigments present in the natural food materials depend on several factors such as the plant cultivar, the geographical origin, the climatic conditions and the time of harvest. There are several permitted pigments in food industry derived from natural sources, which may be used for food coloration. These are curcumin (curcuminoid), lutein (xanthophyll), beta-carotene, bixin/norbixin, capsanthin/capsorubin (carotenoids), betanin (betalaine), carminic acid and carmine (anthraquinones), chlorophyll and copper chlorophyll/chlorophyllin (porphyrins), caramelised sugar and malt extract (melanoidins) and anthocyanins (flavonoids). Anthocyanins have raised a growing interest due to their extensive range of colours.

Elderberry is a potential source of natural food colorant because of its high amount of anthocyanins and its not demanding, "easy-to-grow" characteristics. Elderberry pigments are almost exclusively cyanidin glycosides, from which cyanidin-3-O-glucoside and cyanidin-3-O-sambubioside are the major ones. In addition, cyanidin-3-O-sambubioside-5-O-glucoside and cyanidin-3,5-O-diglucoside were detected as minor compounds (Hong et al., 1990).
In our research, anthocyanin profiles of one Austrian (Haschberg) and four Danish (Sampo, Samocco, Samyl, Samident) elderberry varieties grown in Hungary were investigated during ripening with HPLC-VIS and HPLC-ESI-qTOFMS analytical techniques. The aim of this work is to reveal the compositional differences that are hypothesised to be responsible for the underpinning varying colouring abilities.

2. MATERIAL AND METHODS

2.1. Plant materials

Elderberry fruit samples were harvested in July and August 2012. Five different elderberry (candidate) varieties: Samocco, Samident, Samyl, Sampo, Haschberg were sampled in 2 different growing areas of Hungary: 'Vál' and 'Nagyvenyim'. Hascberg is the leading European variety but other candidate varieties appeared from Denmark and there is little information about their colouring ability yet.
Each sample consisted of subsamples picked from at least 4 bushes and were packed in plastic bags and stored at -18 °C until further analysis. Samples were classified in 6 different maturity status based on visual judgement.

2.2. Analytical methods

Chromatographic separation of anthocyanin components was performed by an HPLC system using 125×4 mm C18 column with 3µm particle size (Dr. Maisch GmbH, Germany). For the elution, 0.5% (v/v) formic acid in high purity water (mobile phase A) and 0.5% formic acid in acetonitrile (mobile phase B) were used as solvents at a flow rate of 0.5 ml/min. Tentative identification of intact anthocyanin components was carried out by an HPLC-ESI-qTOFMS system based on accurate mass analysis (Agilent 1200 HPLC and Agilent 6530 qTOFMS). Quantification of anthocyanins was measured with an Agilent 1200 HPLC system equipped with a variable wavelength UV-Vis detector at 520 nm. External calibration was performed using cyanidin-3-glucoside reference standard (Extrasynthese, Genay, France). Concentrations of all anthocyanin compounds were assessed from peak areas and are given as cyanidin-3-O-glucoside equivalent (CGE) per 100 g of fresh elderberry fruit. Total anthocyanins (TA) were expressed as sum of individual anthocyanin concentrations.

3. RESULTS

HPLC-ESI-qTOFMS system revealed that there three major cyaniding-based anthocyanins were present in the investigated elderberries, namely cyanidin-3-O-sambubioside-5-O-glucoside (*m/z* 743.2020), cyanidin-3-O-sambubioside (*m/z* 581.1419) and cyanidin-3-O-glucoside (*m/z* 449.1076) and some minor components (Fig. 1). It was shown that in general, each anthocyanin compound followed the same kinetics during ripening however, in some cases the abundance of these major anthocyanins are variety and maturity dependent, see Fig. 2. The a) chromatogram shows the 2nd maturity stages of Haschberg variety, the b) chromatogram is the 2nd maturity stage of ‘Sampo’ and the c) chromatogram is the 3rd maturity stage of ‘Sampo’ from the same growing area of Vál.

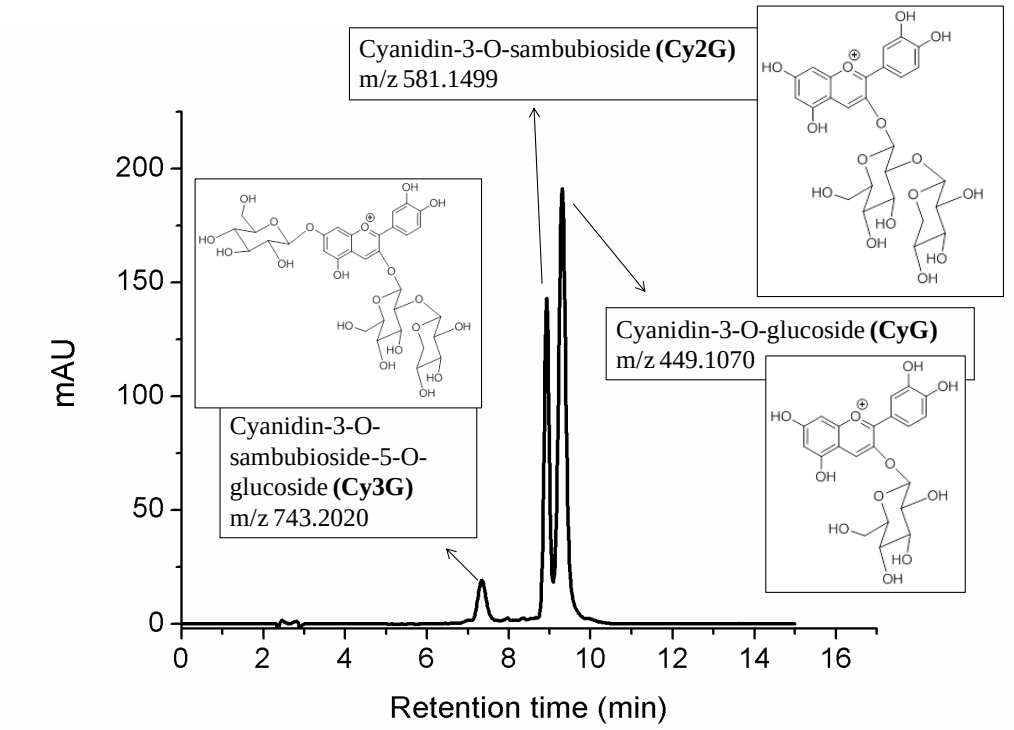
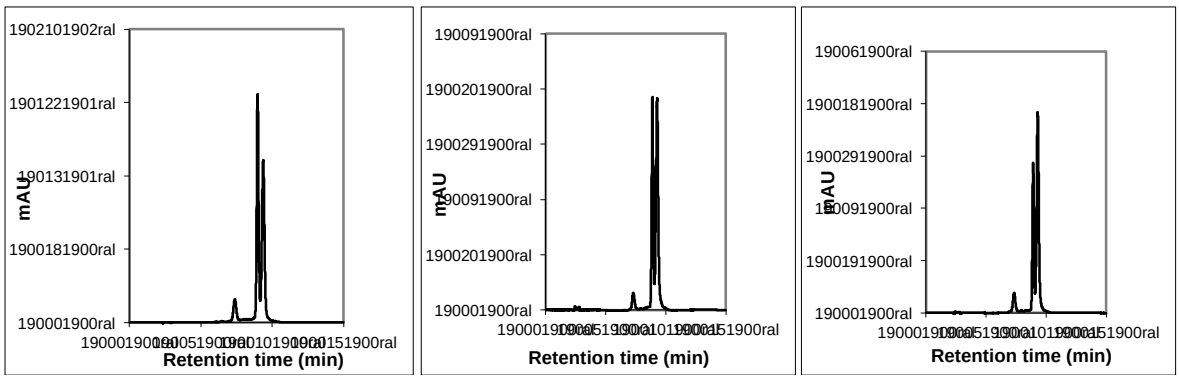


Figure 1: Typical chromatogram of identified anthocyanin components in elderberry extract

The two major anthocyanins in examined elderberries are cyanidin-3-O-glucoside and cyanidin-3-O-sambubioside which is in accordance with other authors (Wu et al., 2004; Kaack et al., 2008).



a) b) c)

Figure 2: Three different anthocyanin profiles in elderberry varieties

Table 1 shows that the major anthocyanin in all berries from the investigated cultivars cyanidin-3-O-glucoside (CyG), accounted for up to half of all determined anthocyanins, approximately. The only exception was 'Samocco', where the major anthocyanin was cyanidin 3-O-sambubioside, accounting for more than 55% of all analysed anthocyanins. The lowest concentration of CyG was detected in 'Samyl' from Vál (288.3 mg CGE/100g FW) and the highest in cultivar 'Samocco' (582.5 mg CGE/100g FW). The lowest amount of cyanidin-3-O-sambubioside (i.e., the anthocyanin with the second highest concentration), was measured in the berries of 'Samident'(273.9 mg CGE/100g FW) and the highest in 'Samocco' (944.2 mg CGE/100g FW), both varieties originated from Vál. Candidate variety 'Samocco' has the highest concentration of total anthocyanin content in both growing areas because

quantity of pigments is 1671.4 mg CGE/100g FW in Vál and 1519.7 mg CGE/100g FW in Nagyvenyim.

Table 1: Concentration of three identified anthocyanins in elderberry fruits

(Candidate) varieties	Growing area	Maximum maturity stage	Cy3G (mgCGE/100g)	Cy2G (mgCGE/100g)	CyG (mgCGE/100g)	TA (mgCGE/100g)
Haschberg	Vál	6th	103.7 ± 19.9	426.8 ± 82.8	345.1 ± 67.7	875.6
Haschberg	Nagyvenyim	4th	77.8 ±7.7	431.1 ± 40.9	516.6 ± 48.3	1025.5
Sampo	Vál	5th	72.0 ± 13.1	304.1 ± 53.6	326.9 ± 57.3	703.0
Sampo	Nagyvenyim	5th	95.1 ± 8.8	456.7 ± 41.6	529.6 ± 47.8	1081.4
Samident	Vál	5th	57.7 ± 9.8	273.9 ± 47.3	289.3 ± 51.4	621.0
Samident	Nagyvenyim	3th	59.8 ± 4.6	406.0 ± 23.6	486.4 ± 24.2	1438.6
Samocco	Vál	5th	144.7 ± 22.85	944.2 ± 145.2	582.5 ± 85.0	1671.4
Samocco	Nagyvenyim	5th	115.0 ± 18.5	822.2 ± 124.6	582.5 ± 88.3	1519.7
Samyl	Vál	6th	68.7 ± 27.45	281.2 ± 113.0	288.3 ± 113.3	638.3
Samyl	Nagyvenyim	6th	87.7 ± 14.9	550.1 ± 91.3	364.7 ± 60.8	1002.4

4. CONCLUSION

Elderberry fruit is predominantly used for food coloration, therefore (candidate) varieties with higher anthocyanin content are particularly suitable for commercial growing. Among the investigated cultivars candidate variety 'Samocco' has the highest total anthocyanin content in 5th maturity stage in both growing areas. Abundance of anthocyanin component can be a variety dependent property because anthocyanin profile of 'Samocco' was different from the rest of the samples. Furthermore regional effects in anthocyanin concentration can be observed for all varieties/candidate varieties especially in the case of 'Samident'. These findings may suggest that the varying coloring capabilities, especially the varying stability of coloring power of different elderberry varieties might be in relation with the variety dependent anthocyanin profiles. In other words, "total anthocyanin" concentrations of elderberry extracts alone, might not provide exhaustive information on the quality and thus the value of the elderberries intended to use as natural colorants.

It should be noted however, that natural food colorants are usually used in the form of powder, concentrate or extract, the anthocyanin profile of these products may be also influenced by the interventions during processing (pressing, enzymatic treatment, heat treatment etc.). It means, these aspects should be also taken into account when the coloring power of the end-product is under discussion, however further research is needed in this field and these questions are yet to be addressed.

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DEVELOPMENT OF AN OWLS-BASED IMMUNOSENSOR FOR THE DETECTION OF AFLATOXIN M1

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SUMMARY

An immunoreaction-based antigen determination method was investigated for the detection of aflatoxin M1 (AFM1), which is the hydroxylated metabolite of aflatoxin B1 (AFB1). This mycotoxin may be found in milk and milk products obtained from livestock that have ingested contaminated feed. Quantitative analysis of AFM1 was carried out using indirect (competitive) immunoassay method which, can be used for low weight molecules. The real-time measurement was done with Optical Waveguide Lightmode Spectroscopy (OWLS) technique. After the optimization of the measuring system (determination of the optimal concentration of the immobilized AM1-protein conjugate, determination of the AFM1 antibody content of the samples etc.) real samples were also examined. The presented competitive immunoassay method shows a good sensitivity to AFM1 in the range of 0.001-0.1 ng mL^{-1} with the detection limit of 0.0005 ng mL^{-1} in 100 fold diluted milk samples.

1. INTRODUCTION

The selective detection of low concentrations of aflatoxin M1 (AFM1) is important for food safety, since it belongs to the most toxic mycotoxin class. There are currently several accepted measuring techniques available for determining AFM1. These include enzyme-linked immunosorbent assays (ELISAs) (Rastogi, 2004) and traditional analytical methods, such as high-performance chromatography (HPLC) and thin-layer chromatography (TLC) (Sydenham, 1996). In recent years biosensors have come to the front as alternative tools in the field of AFM1 detection. As for biosensors, some DNA-based (Siontorou, 1998; Dinckaya, 2011) or cell-based (Larou, 2013) sensors with electrochemical detection have been developed. However, most of the reported bioanalytical tools are immunosensors, requiring enzyme-labelling (Badea, 2004; Micheli, 2005; Parker, 2009). Their limits of detection (LOD) for AFM1 varies between 11 and 40 ng kg^{-1} in milk samples. Our aim was to achieve lower LOD by developing a label-free competitive immunoassay based on optical waveguide lightmode spectroscopy (OWLS) technique.

2. MATERIALS AND METHODS

2.1. Materials

AFM1, γ-aminopropyltriethoxysilane (APTS) and tris(hydroxymethyl)aminomethane (Tris) were purchased from Sigma-Aldrich (MO, USA), polyclonal rabbit anti-AFM1 serum against aflatoxin M1-bovine serum albumin (AFM1-BSA) conjugate from Agrisera (Sweden), coating antigen of aflatoxin M1–horseradish peroxidase (AFM1-HRP) conjugate from R-Biopharm (Germany), 25% aqueous solution of glutaraldehyde from Merck (Germany). All other reagents were of analytical grade and double distilled water was used in this research. The milk (1.5% low-fat UHT) was purchased from a local store.

2.2. Instrumentation

OWLS measurements were performed using amino functionalized integrated optical waveguide sensors (chips) type OW 2400 (MicroVacuum, Budapest, Hungary). The chips contain optical grating on the top of the glass support. The sensor output was read with an OWLS120 instrument BioSense 2.6.8 software (MicroVacuum Ltd, Budapest, Hungary). The

temperature control unit (MicroVacuum Ltd, Budapest, Hungary) was connected to the OWLS system. All the experiments were carried out in flow injection analyzer system containing a syringe pump (model: NE-1000, NY, USA), and built in injection valve (model: Rheodyne 9725, CA, USA) fitted with a 200 µL sample loop.

2.3. AFM1-HRP immobilization and measuring method

Before the measurements, amino functionalized SiO_2–TiO_2 sensor surface was prepared (Trummer, 2001). The silanization was followed by the activation of the sensor surface with 200 µL of 2.5% glutaraldehyde, which was performed immediately before the AFM1-HRP immobilization in flow through system. The surface was then washed with distilled water. After stable baseline was obtained, AFM1-HRP conjugate solution was injected in an appropriate dilution. Finally, the distilled water was changed to the reaction buffer of 42 mmol L^{-1} Tris (pH 7.4). At the end of the immobilization 10 mmol L^{-1} HCl was injected to wash the surface. Followed by the baseline stabilization (approximately 10 min), the system was ready for sample measurement. Upon the injection of the corresponding samples, 10 mmol L^{-1} HCl was injected in order to dissociate the antigen-antibody complexes, thus regenerating the sensor. The applied flow rate, temperature were 0.12 mL min^{-1} and 24°C, respectively, during all the measurements.

2.4. Sample preparation

Standard AFM1 solutions and milk samples were prepared for indirect measurements. Milk was spiked with different quantities of mycotoxins, simply filtrated (particle retention of 5–13 µm) and diluted 50 fold (42 mmol L^{-1} Tris, pH 7.4) before being measured. Standard solutions or milk samples were then mixed with antibody solution of appropriate concentration. After 3 min incubation (24°C), the mixture was injected to the sensor surface.

3. RESULTS

3.1 Competitive measurement of AFM1

For competitive measurements, AFM1-HRP conjugate was immobilized on the sensor surface as detailed in 2.3. The immobilization was followed by the examination of standards or milk samples. Standard solutions or milk samples were mixed with antibodies of the appropriate concentration in 1:1 ratio. The mixture was incubated at 24°C for 3 min. After that, it was injected into the system. Only the antibodies remaining free in the sample mixture can bind to the antigens immobilized. For that reason, the amount of antibodies binding to the surface is inversely proportional to the quantity of the antigen in the standard solutions.

3.2 Determination of the amount of the applied polyclonal serum

The determination of the amount of the applied polyclonal serum is substantial in the case of indirect measurements. For this examination, 7500 fold dilution of AFM1-HRP conjugate was immobilized on the surface, as detailed in 2.3. The sensor responses of the antisera at different concentrations (500×; 1000×; 2000×; 4000×; 10000× fold dilutions) were compared. As it can be seen on Figure 1, the signal was unstable at 10000× dilution of the antisera. For higher protein content of antisera (4000×; 2000×; 1000× fold dilutions) the signals obtained were stable. Furthermore the methods seem to be sensitive enough to detect low amounts of AFM1. At 500 fold dilution, although signal responses were high, the surface of the sensor was saturated. Based on the results, 4000× fold dilution of the antisera was used for the competitive measurements.

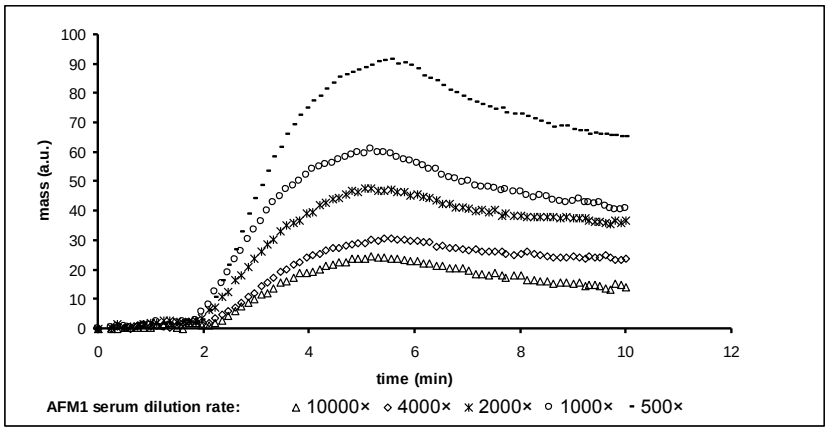

Figure 1: Sensor responses of AFM1 antisera at different dilutions
(7500× diluted AFM1-HRP conjugate immobilized; 42 mmol L^{-1} Tris, pH 7.4; 0.12 mL min^{-1} flow rate, 24°C)

3.3 Determination of the optimal antigen conjugate concentration for immobilization

In order to define the optimal AFM1-HRP coating, different dilutions (10000×; 7500; 5000× dilutions) were immobilized on the sensor surface and tested with competitive AFM1 measurements as follows. The 2000× fold diluted antisera was mixed with the appropriate concentration of the AFM1 standard solutions (0.001-100 ng mL^{-1}) in the ratio of 1:1. After 3 min incubation time, the solution was injected onto the sensor surface. Figure 2 shows the effect of the concentration of AFM1–HRP conjugate immobilized on the sensor surface. Using 5000× diluted conjugate, the sensor shows a narrow dinamic measuring range for AFM1 (0.01-1 ng mL^{-1}). At higher AFM1 concentrations, the sensor became unstable. Applying the 7500 fold diluted conjugate, the dinamic measuring range was between 0.001-100 ng mL^{-1} and the standard deviations were the lowest (±0.3-0.5) comparing to the other two sensors (±0.4-1.2). Although the 10000 fold diluted antisera showed the best sensitivity in the range of 0.001-0.1 ng mL^{-1} AFM1, the responses for higher AFM1 concentrations became unstable. Taking the results into consideration, 7500 fold diluted AFM1-HRP conjugate was immobilized for further competitive measurements.

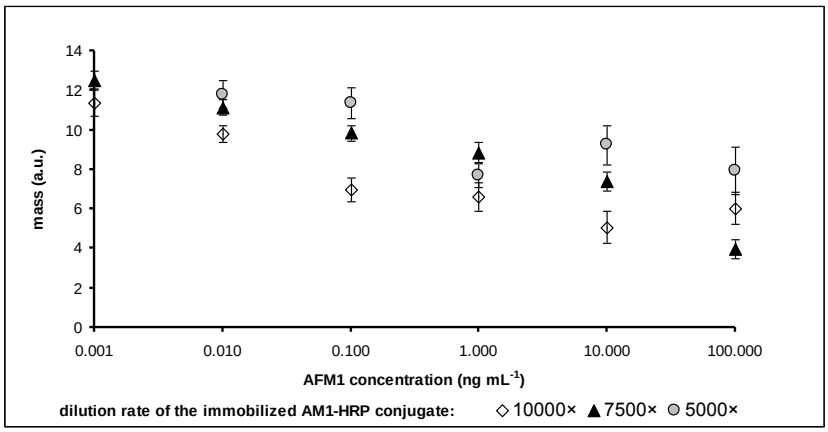

Figure 2: Sensor responses of different dilutions of AFM1-HRP conjugate immobilized on the sensor surface
(4000× diluted AFM1 antisera; 42 mmol L^{-1} Tris buffer, pH 7.4; 0.12 mL min^{-1} flow rate, 24°C)

3.3 AFM1 determination in milk samples

The above detailed examinations were followed by the analysis of milk samples (sample preparation in 2.4). Milk was spiked with different concentrations of AFM1 (0.01-1000 ng mL^{-1}), filtrated and diluted 50 fold with buffer (42 mmol L^{-1} Tris, pH 7.4). These samples were then mixed in the ration of 1:1 with aliquots of antiserum diluted 2000 fold. After the dilution procedure, the AFM1 concentrations in the 100 fold diluted milk samples were between 0.0001 and 10 ng mL^{-1}, whereas the antisera was 4000 fold diluted. Finally, these samples were injected into the OWLS system after 3 min incubation time. A typical calibration curve of spiked milk samples is shown in Fig. 3. The dinamic measuring range was found between 0.001 and 0.1 ng mL^{-1} (1-100 ppt) in 100 fold diluton of milk samples. The improved OWLS-based method is about one order of magnitude more sensitive than those, obtained with similar competitive immunosensors using electrochemical detection (Badea, 2004; Micheli, 2005; Parker, 2009).

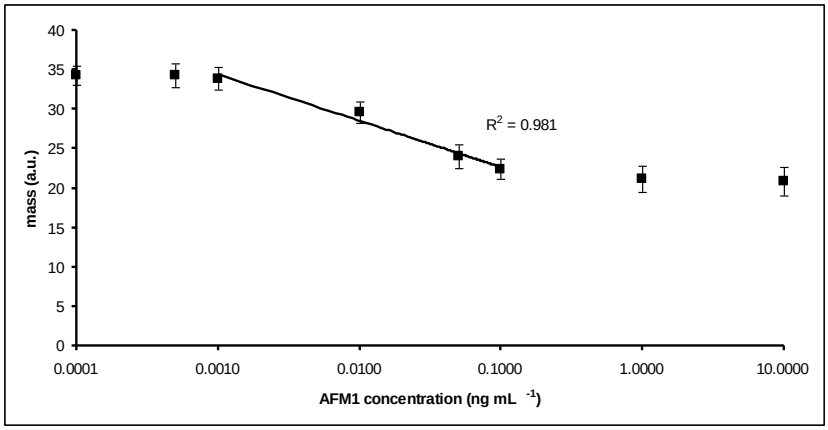

Figure 3: Calibration curve of spiked 100× diluted milk samples
(4000× diluted AFM1 antisera; 7500× diluted AFM1-HRP conjugate; 42 mmol L^{-1} Tris pH 7.4; 0.12 mL min^{-1}; 24°C)

4. DISCUSSION

The elaboration of an indirect method for AFM1 detection has been presented in this work based on non-labelled OWLS detection. After the optimization of the major measuring parameters, such as immobilized antigen dilution (7500× diluted AFM1-HRP conjugate) and the applied antiserum dilution (4000× diluted), spiked milk samples were examined. AFM1 can be detected in the range of 0.001 – 0.1 ng mL^{-1} in 100 fold diluton of milk. According to the results, it can be concluded that this label-free immunosensor is applicable for the quick determination of AFM1 contamination in milk samples.

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EFFECT OF THE HEAT TREATMENT ON THE SURVIVAL OF AERIBACILLUS PALLIDUS SPORES ISOLATED FROM CANNED CORN

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SUMMARY
The aim of this research was to observe thermal inactivation of Aeribacillus pallidus spores at four different temperatures (101.2 0C, 104.2 0C, 107.2 0C and 110.2 0C) and in two different heating media (water and corn juice). The decimal reduction times (D-values) were calculated by linear regression analysis (D=-1/slope of a plot of log surviving cells versus time). The D-values for A. pallidus spores in water ranged from 7.0 min (101.2 0C) to 6.4 min (110.2 0C). The D-values for A. pallidus spores in corn juice ranged from 13.4min (101 0C) to 6.7 min (110.2 0C). In most of the experiments R value was > 0.90. The results obtained from thermal destruction studies show that A. pallidus spores are more resistant to high temperatures in canned corn juice than in water. Future research should be done to observe the influence of intrinsic and extrinsic factors on growth and survival of A. pallidus spores and vegetative cells.

1. INTRODUCTION

Microbial spoilage contributes to the vast amount of food that is wasted and the associated financial losses (Kantor et al., 1997). Spoilage of heat treated food products usually provoked by spore-forming bacteria, as a result of resistance of spores to high temperatures commonly used to preserve foods. Canned food products generally undergo spoilage by thermophilic bacilli. Denny (1981) had demonstrated that thermophilic bacteria were the prime cause of spoilage of canned corn.

In recent years there was an evidence of contamination of canned food (especially canned corn) products by new spore forming, thermophilic bacteria from the genus Aeribacillus (previously Geobacillus). Members of the genus Aeribacillus are aerobic, thermophilic, alkalitolerant, motile, Gram-positive rods (0.8–0.962–5 nm) that occur singly, in pairs or in chains. The reason of spoilage of ready canned corn by A. pallidus can be the improper performance of thermal processes due to incorrect relation between heating time and temperature. For that reason, investigation of heat resistance of A. pallidus spores and determination of decimal reduction time (D-value) is a high concern for canning industry. In comparison with other thermophillic bacteria from the genus Geobacillus, there are no reported D-value data for A. pallidus in canned corn products.

Therefore, the aim of this study was to determine the influence of heat treatment and sanitizers on survival of spores of Aeribacillus pallidus.

2. MATERIALS AND METHODS

2.1. Bacterial strains

In this study, Aeribacillus pallidus cultures obtained from Department of Microbiology and Biotechnology of Corvinus University of Budapest were used. The strains were isolated from spoiled canned corn products. Isolation and storage of A. pallidus cultures was done using Casein-peptone Soymeal-peptone Agar (CASO agar, Merck).

2.2. Preparation of spore suspensions

Agar slants of A. pallidus cells were flooded with sterile distilled water. Than 2 ml of suspension was transferred on surface of CASO agar in 200 mm diameter Petri dishes.

Inoculated Petri plates were incubated at 55 °C for 48 hours and after were transferred to a refrigerator and stored at 15 °C for 72 hours. Spores then were collected by scrapping the surface of the agar with sterile metal spatula and suspended in sterile distilled water and washed three times by centrifugation (4000 x g for 10 minutes). Spore suspensions were stored at 4 °C until they were used. Number of spores in suspension was determined by pour plating method and it was 8x109cfu/ml. Suspensions were diluted to obtain approximately 8x107cfu/ml. Activation of spores was done by heating spore suspension in water bath at 80 °C for 10 minutes.

2.3. Heat treatment analysis

Water and canned corn juice were used as a heating media. Heating experiments were carried out in small glass vials. After filling with 2.5 ml of spore suspension, vials were sealed in gas burner flame. Thermal inactivation was performed in temperature controlled oil bath (Memmert, Model ONE 7, Germany). The samples were heated in the oil bath at the temperatures of 101°C, 104 °C, 107 °C and 110 °C. The sample temperature was monitored continuously using Testo 110-1 channel NTC Thermometer with needle type sensor. Triplicate samples were removed from the bath every 3 minutes, at 0, 3, 6, 9 and 12 minutes. After removal, the samples were immediately immersed into cold water. The viable spores were counted by triplicate plating on CASO agar and incubated at 50 °C for 2 days.

2.4. Calculation of D10-values

D10-values were calculated using the average slope (D10 = -1/slope) for each temperature treatment.

2.5. Statistical analysis

Each experiment was done in triplicates. For determination of D-values linear regression analysis were done (using Excel software).

3. RESULTS

Thermal inactivation tests of A. pallidus spores at four different temperatures (101.2 °C, 104.2 °C, 107.2 °C and 110.2 °C) and in two different heating media (water and corn juice) have been performed. The decimal reduction times (D10-values) were calculated by linear regression analysis (D10 = -1/slope of a plot of log surviving cells versus time).

As an example, survival curves of A. pallidus spores in different heating media at 104.2 °C are shown at Figure 1.

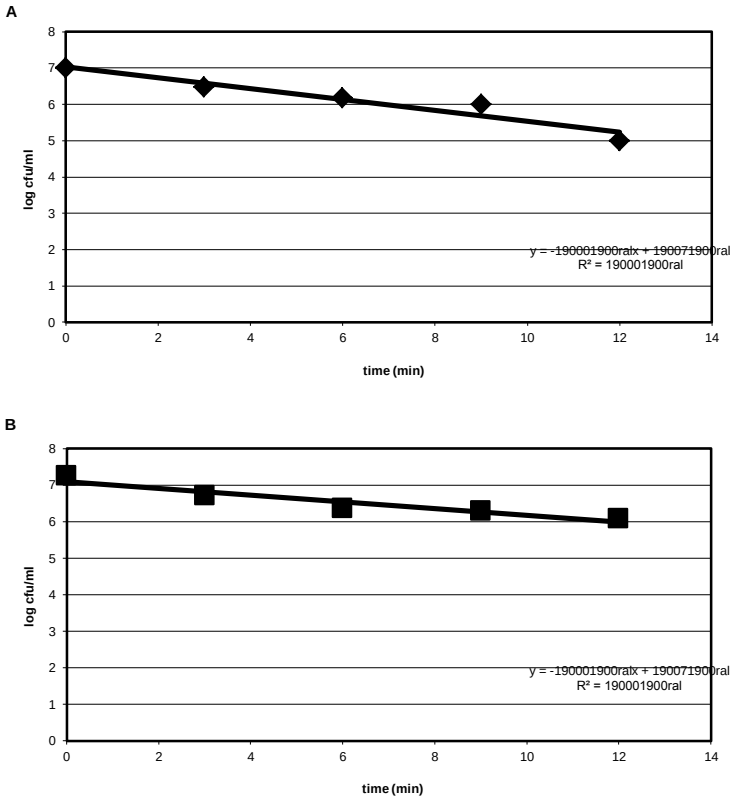

Figure 1: Survival curves of A. pallidus spores at 104.2 °C heating temperature in sterile water (A) and in canned corn juice (B)

The D10-values for A. pallidus spores in water ranged from 7.0 min (101.2 °C) to 6.4 min (110.2 °C). The D-values for A. pallidus spores in corn juice ranged from 13.4 min (101 °C) to 6.7 min (110.2 °C). In most of the experiments R2 value was > 0.90. The results obtained from thermal destruction studies show that A. pallidus spores are more resistant to high temperatures in canned corn juice than in water.
Table 1. summarizes the D-values obtained at different heating temperatures in water and in corn juice.

Table 1: D10-values of A. pallidus spores at different temperatures in water and in corn juice

Heating temperature (°C)	D10-value (min)	
	Water	Canned corn juice
101.2	7.00	13.44
104.2	6.82	10.79
107.2	6.70	10.85
110.2	6.37	6.68

4. CONCLUSION

Considering results obtained from this preliminary study it can be concluded that resistance of Aeribacillus pallidus to thermal treatments depends on heating media. In canned corn juice A. pallidus spores show higher resistance than in water. It can be explained by presence of number of nutrients in canned corn juice which provide good conditions for survival of damaged spores. Furthermore, presence of small particles in corn juice can serve as an additional protective barrier for spores against high temperatures. Future research should

be done in wider temperature range to observe the influence of intrinsic and extrinsic factors on growth and survival of A. pallidus spores and vegetative cells.

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BIOACTIVE COMPOUNDS OF TOMATO AS AFFECTED BY WATER STRESS

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SUMMARY

The objective of this study was to investigate the influence of water stress on the composition and content of phenolic compounds and carotenoids in different tomato varieties using the HPLC/DAD-UV technique and reverse-phase (RP) chromatographic columns. It was found that response of tomato to water stress is affected by genetic factor and seasonal environmental variations. In general, 100% irrigation yielded tomatoes with the lowest level of carotenoids and polyphenols. In 2012, when the temperature and number of sunny hours were at record level, the non-irrigated plants of cultivar Strombolino gave tomatoes with significantly higher level of carotenoids and phenols than that of the other cultivars.

1. INTRODUCTION

The tomato is one of most important vegetables worldwide. Its consumption either as fresh fruit or as processed product is higher than for all other fruits and vegetables. The tomato has been reported to be an important source of bioactive compounds such as carotenoids, tocopherols and phenolic compounds, in the human diet (Abushita et al., 2000; Slimesterd and Verheul, 2009). Among carotenoids, tomatoes contain high level of biologically active lycopene and, in some varieties, considerable amounts of β-carotene (Kotíkova et al., 2011). Phenol compounds are a group of secondary plant metabolites which play key roles in plant protection, including defense against pathogens and protection against UV-B radiation, and contain several chemical structures, from simple molecules to highly polymerized forms. Tomatoes have been found to contain high amounts of cinnamic acids (caffeic-, chlorogenic-, ferulic acid) and their derivatives in addition to some flavonoids (rutin, quercetin) (Slimesterd and Verheul, 2009). Polyphenols are highly reactive antioxidants reducing the risk of several chronic diseases (Anderson and Markham, 2006).

During the last decades there were dramatic environmental changes including water deficiency, salinity and warming. Our main target in the present work was to study the effect of water stress on the main bioactive compounds of tomato fruits from different varieties.

2. MATERIALS AND METHODS

2.1. Plant material and treatments

Three tomato varieties were grown in the experimental fields of the Szent István University, Gödöllő, Hungary. They utilized different water supply quantities (50%, 75%, 100% irrigated and control non-irrigated) respectively during the 2010, 2011 and 2012 vegetable growing seasons. Drip irrigation water was dispensed according to the air temperature. Whole tomatoes were homogenized with a blender and the samples were stored at -20°C until analysis.

2.2. Phenolic components

Phenolic components were extracted from 10 g sample with 30 ml of water-methanol-acetic acid (25:75:2) solution. The filtrated extracts were further cleaned by passing through a

0.45-mm HPLC syringe filter before injection into HPLC column for analysis of phenolic compounds.

Chromatographic separation of phenols was performed on an EC Nucleodur Sphinx RP, 3 mm, 150x4.6 analytical column using gradient elution. The mobile phase was [A] 1% formic acid in water, [B] acetonitrile.

Peaks of phenol components were identified by comparing their spectral characteristics and retention with those of available standards. Integration was done at the maximum absorption of each compound. Phenols were detected at 280, 320, 355 nm.

2.3. Carotenoid components

Three grams of tomato were extracted, after crushing in a crucible mortar in the presence of quartz sand, with 20 ml methanol. The mixture was then shaken with 10 ml methanol and 50 ml 1,2-dichloroethan for 10 min. Bi-distilled water was added to separate the polar and non-polar phases (Daood et al., 1989). The pigment-containing solvent was dehydrated over anhydrous Na_2SO_4 and evaporated at 40 ^{0}C to dryness under vacuum. The residues were re-dissolved in 5 ml HPLC grade acetone. After filtration through filter paper and a PTFE 0.45 μm syringe filter, the clear solution was injected into the HPLC column.
Separation of carotenoids was performed on cross-linked base-deactivated reversed-phase EC 150/4.6 Nucleodur C18, ISIS 3μm column with gradient elution of water in acetone (Daood et al., 2013). Peak identification was based on comparison of retention times and spectral characteristics with those of pure standards.

2.4. HPLC instrument

A Waters Alliance system consisting of a Model 2695 separation module and a Model 2996 photodiode-array detector was used for the chromatographic analysis of the tomato phenols and carotenoids. The system was operated by Empower software.

3. RESULTS

3.1. Effect on phenols

The results of the HPLC analyses of phenolic acids and polyphenols are shown in Figure1, 2, 3 and 4. The main phenolic acids were identified as chlorogenic- and caffeic acid and their derivatives. Rutin was found to have of the largest amount among polyphenols.

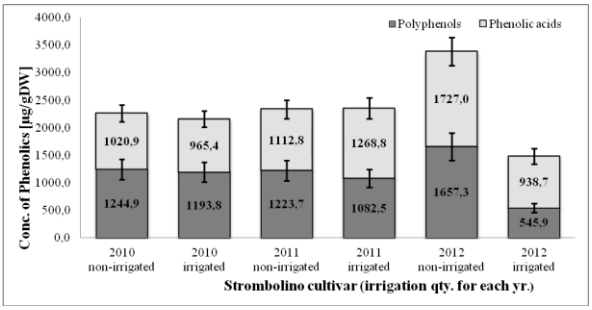

Figure1: Changes in phenolic compounds as an effect of irrigation and water stress in Strombolino cultivar (2010-2012)

The content of both groups of phenols in tomato of Strombolino variety was not affected by irrigation during the seasons 2010 and 2011, while significant difference was found between fruits of irrigated and non-irrigated plants in 2012 when the season temperature and number of sunny hours were extremely high.

The highest concentration of phenols was also measured in fruits of non-irrigated plants from other varieties cultivated in two seasons. Uno Rosso variety contains significantly higher concentration of phenolic compounds in 2012 than in 2011. Furthermore, there was no

significant difference between 50% and 75% irrigation in their effect on the content the phenolic compounds in tomato fruits. In every season the cherry type tomato Strombolino contained 1,5-2 times higher amount of polyphenols than the normal types.

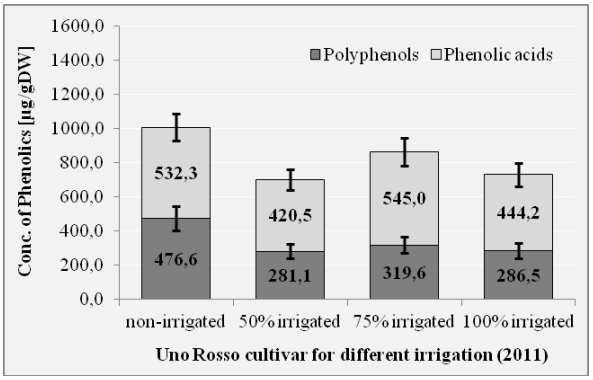

Figure2: Changes in phenolic compounds as an effect of irrigation and water stress in Uno Rosso cultivar (2011)

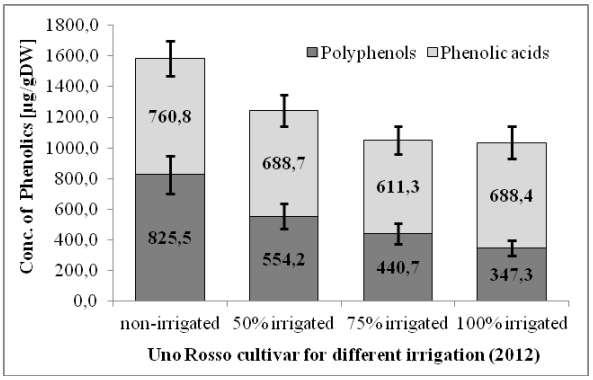

Figure3: Changes in phenolic compounds as an effect of irrigation and water stress in Uno Rosso cultivar (2012)

3.2. Effect on carotenoids

In the evaluation we focused on lycopene as being the dominant carotenoid compound and because of its high biological activity. The highest level and proportion of lycopene was found in Strombolino variety making it of special interest the nutritional and tomato processing points of view (Figure5). Like that of phenols, biosynthesis of carotenoids was activated in Strombolino in 2012 as an effect of high temperature and number of sunny hours in the season. The increase in phenols and carotenoids may also be linked to the effect of meteorological parameters on dry matter content of tomato. As regards the effect of irrigation on carotenoid content of tomato, the results of two years indicated that 75% irrigation results in tomatoes with significantly lower carotenoid concentration particularly in Uno Rosso variety. Other irrigation levels did not show any significant differences (Figure6,7). However, in 2012 lycopene content of fruits from 100% irrigated Triple Red variety (Figure8) was significantly higher than that found in fruits from non-irrigated plants.

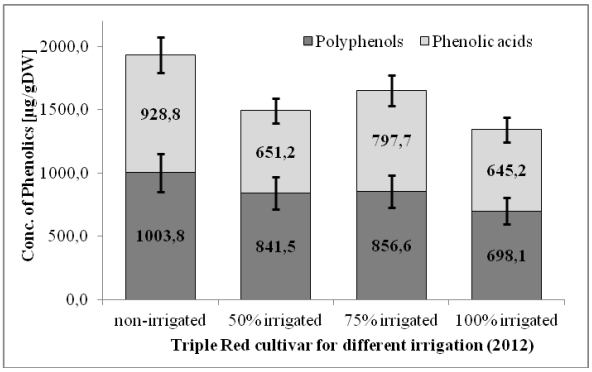

Figure4: Changes in phenolic compounds as an effect of irrigation and water stress in Triple Red cultivar (2012)

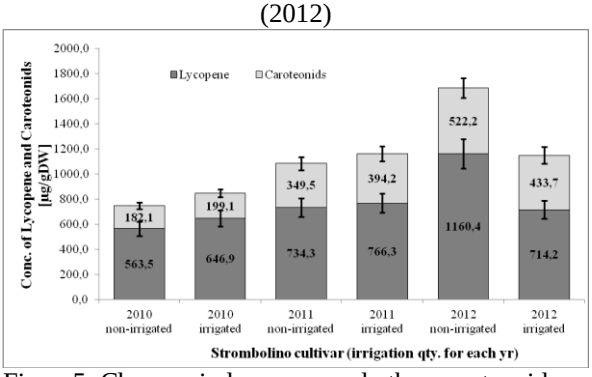

Figure5: Changes in lycopene and other carotenoids as an effect of irrigation and water stress in Strombolino cultivar (2010-2012)

These results show that response of different varieties to water stress is different. It is therefore important to optimize irrigation to produce tomatoes with high content of bioactive nutrients such as carotenoids especially lycopene, a compound responsible for the red color and contributes substantially to the biological activity of tomatoes. The genetic, technological and environmental factors were found to influence, to a significant extent, the antioxidant content and nutritive value of tomato products (Dumas etal., 2003; Raffo et al., 2006; Stewart et al., 2000; Sánches-Rodríguez et al., 2012).

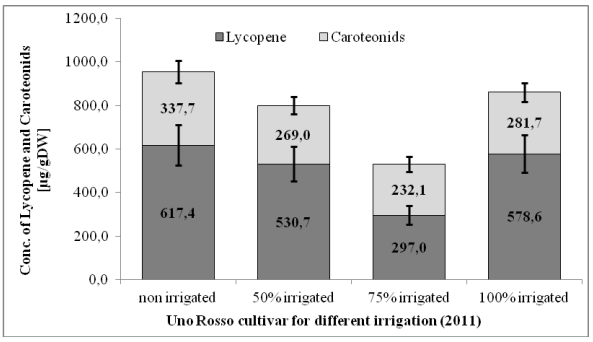

Figure6: Changes in lycopene and other carotenoids as an effect of irrigation and water stress in Uno Rosso cultivar (2011)

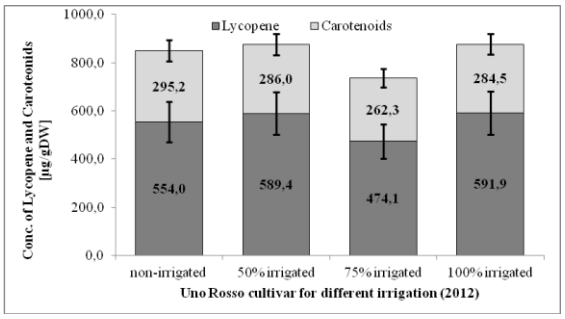

Figure7*:* Changes in lycopene and other carotenoids as an effect of irrigation and water stress in Uno Rosso cultivar (2012)

Light, temperature and other climatic conditions have been found to have an effect on bioactive compounds such as flavonoids and carotenoids in tomatoes. In general light and high temperature have promoted biosynthesis of bioactive compounds (Slimestred and Verheul, 2009). As concerns the effect of water stress many researches have linked the promotion of biosynthesis of some phytochemicals in tomato to its effect on mineral absorption and limitation in nitrogen supply (Stewart et al., 2001).

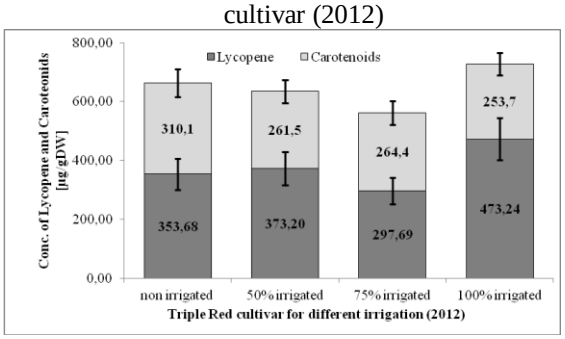

Figure8: Changes in lycopene and other carotenoids as an effect of irrigation and water stress in Triple Red cultivar (2012)

ACKNOWLEDGEMENT: *The authors would like to thank the National Development Agency (NFÜ) for financial support under project grant no. TECH-09-A3- 2009-0230, USOK2009*

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DIFFERENT AROMA EXTRACTION METHODS FOR THE ISOLATION OF FOOD VOLATILES

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SUMMARY

The composition of volatile fraction of red pepper powder was examined by distillation methods and headspace analysis. Unique fragrance patterns were obtained by each processes. Apparent differences were experienced regarding the number and intensity of the extracted aroma components: distillation techniques proved to be more efficient than headspace analysis. In case of the latter method, polarity of the SPME fibers was an important parameter as well in terms of extraction. The formation of thermal degradation products are often attributed to the application of distillation techniques. The majority of these volatiles appeared in the headspace of the products also, in spite of the lower temperature. On the basis of the extracts rich in odour-active fragrance constituents, distillation methods appear more suitable for examining aroma composition.

1. INTRODUCTION

The dried and ground fruit of red pepper (*Capsicum annuum* L.) is a traditional and frequently used condiment in Hungarian cuisine. This spice is well-known for its characteristic, pleasant aroma and bright colour. A number of authors have been investigated the aroma constituents of this spice, since the knowledge of the volatile composition of red pepper may be an important tool in determining identical variety or in verification of origin. Sample preparation is a crucial step in aroma analysis, since the extract has to represent the characteristic features of the original sample. The aim of this study was to compare the fragrance composition of this important Hungarian spice applying different sampling procedures.

2. MATERIALS AND METHODS

2.1. Samples

The examined sweet red pepper powder of the ’special’ quality class was purchased from a local market. Prior to sampling, the powder was homogenized in a glass beaker.

2.2. Sample preparation

For solid phase microextraction, the sample was put into a special headspace flask equipped with a screw-cap syringe valve and it was placed to a 60 °C water bath. Four different fibres were applied to the extraction of the volatiles: 100 µm Polydimethylsiloxane (PDMS), 85 µm Polyacrylate (PA), 75 µm Carboxen-Polydimethylsiloxane (CAR-PDMS) and 65 µm Polydimethylsiloxane-Divinylbenzene (PDMS-DVB). The fibers were conditioned before the extraction, following the recommendations of the manufacturer. After 20 minutes of extraction, the fiber was transferred to the gas chromatograph. For distillation methods, the red pepper powder was placed into a round-bottom flask and was completed with distilled water and NaCl. Undecanol-1 was applied as internal standard in the course of the processes. For steam distillation, n-hexane was applied as an organic solvent, while in simultaneous distillation extraction the volatiles were extracted with pentane. Volatiles from three subsequent distillation were collected into the same solvent. Prior to the instrumental

measurement, the extract was frozen to release from water, then it was concentrated and injected into the GC-MS.

2.3. Instrumental analysis

The analysis was performed on a GC-MS system (HP 5890/II – HP 5971A MSD). The instrument was equipped with a 60 m AT-WAX fused silica capillary column. The temperature programme was the same every time. In case of distillation methods, the injector was operated in splitless mode at 250 °C, with a 100:1 split ratio and 0,1 min splitless time. In SPME analysis, the desorption time was 15 min, and the splitless time had beeen increased to 1 min. The detector was run in electron impact mode (70 eV) at 280 °C. Helium was used as a carrier gas.

3. RESULTS

The aroma profile of the red pepper powder was depended on the sampling method applied: the number and the intensity of the individual fragrance constituents were quite different (Fig. 1).

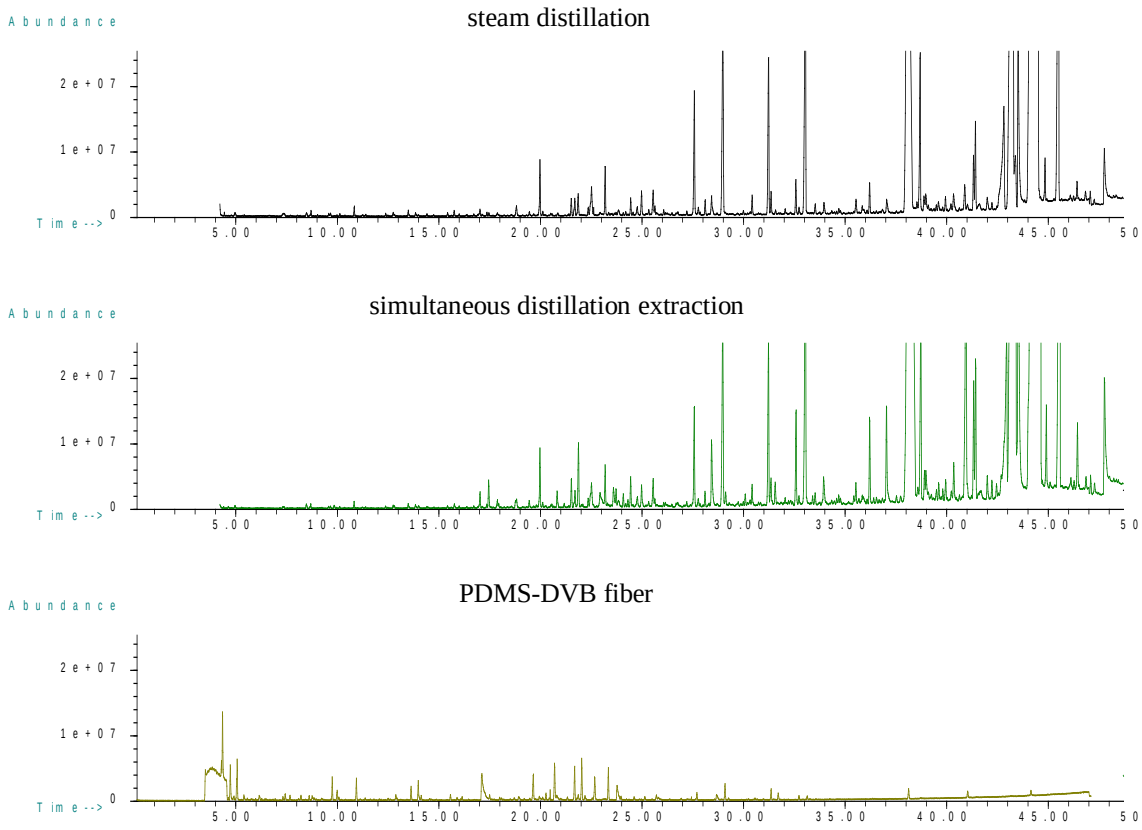

Figure 1: The sensitivity of the distillation methods and the best SPME fibre

Polar Polyacrylate and nonpolar PDMS fibres proved to be the least effective with their 23 and 46 aroma constituents absorbed. Both of them were particularly sensitive for methyl acetate and acetic acid. Among the terpene compounds, carotenoid degradation products like safranal, geranyl acetone and *β*-ionone were predominant on the fibres. Regarding their share from the total aroma, nonpolar PDMS fiber showed the greatest affinity for these odour-active

components: besides carotenoid degradation products some sesquiterpenes were absorbed on the fibre also. Owing to their combined stationary phases, bipolar fibres were more efficient in adsorbing spice volatiles: the number of the fragrance components were 67 and 107 in case of Carboxen-PDMS and PDMS-DVB fibres, respectively. Carboxen-PDMS bipolar fibre was particularly sensitive to the oxygen-heterocycles, acids with low carbon number and acyclic alcohols, aldehydes, ketones but it showed the lowest affinity for terpenes with its 5 detected constituents and negligible ratio. From the fibres used, PDMS-DVB proved to be the most efficient considering the number of adsorbed compounds. This bipolar phase succeeded in detecting numerous odour-active mono- and sesquiterpenes, N-, S-, and O-containing volatiles, and benzene constituents. A number of less fragrant alcohols, aldehydes, ketones and hydrocarbons adsorbed on this fiber also. Distillation methods were more effective in extracting fragrance constituents, 116 and 143 components were detected in these extracts arising from the steam distillation and simultaneous distillation extraction processes. These extracts contained all the fragrance substances that have been expelled from the plant material during boiling. The huge difference among the intensities of the extracted volatile compounds (Fig. 2) was induced mainly by the sensitivity of the distillation methods to fatty acid esters of great molecular weight and low volatility. These properties prevent their sorption on the SPME fibres, thus the majority of them appear on the chromatograms of the distillation methods alone.

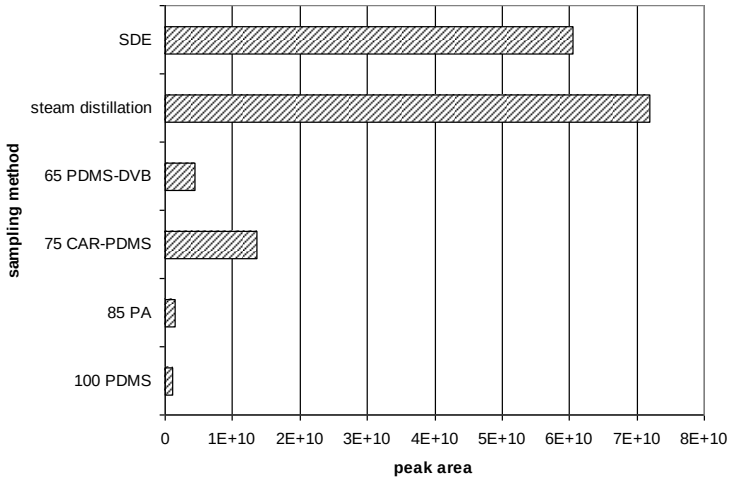

Figure 2: Total peak areas applying different sampling methods

Artefact production is often mentioned as a potential disadvantage of the distillation methods (Kataoka et al., 2000; Marsili, 2002; Riu-Aumatell et al., 2011). The precursors involved in these thermal processes are generally carbohydrates, amino acids and unsaturated fatty acids. These substances are all present in the red pepper pericarp. The majority of their degradation products like furan compounds, pyrazines and some aldehydes were detected by headspace analysis also, despite of the lower temperature and shorter sampling period – moreover, certain O-heterocycles were adsorbed by the SPME fibres alone. Consequently, these fragrance substances are important constituents of the volatile fraction of red pepper powder. Fragrance compounds regarded as artefacts might be among the least odour active esters, fatty acids and hydrocarbons of great molecular mass. Explanation of the origin of heat-induced volatiles in red pepper powder is a quite different task as the plant material is subjected to heat upon a great number of occasions during processing. On the other hand, these odour constituents are integral parts of the characteristic red paprika aroma perceived by the consumers, since this spice is used traditionally in cooked, boiled and smoked dishes.

4. CONCLUSIONS

Selecting the proper sampling method for the examination of food volatiles is not a simple task. Our results clearly prove that no sample preparation method generally applicable exists, the applied procedure must fit the analytical task to be solved. To detect the really fragrant terpene and sesquiterpene constituents might be responsible for the *cv*. characteristic scent composition of red pepper, SPME sampling performed with PDMS-DVB fibre might do the job. To detect fragrance constituents that may indicate the maturity state of the fruit – mainly the alcohols and aldehydes of six carbon number (Luning et al., 1994; Pino et al., 2006) –, Carboxene-PDMS fibre might be sufficient. If the main task is to characterize the fragrance property of the red pepper powder, the detection of as many compounds as possible is necessary. In this latter case only one of the two distillation methods is efficient enough to gather the required information answering the question.

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MICROBIOLOGICAL STUDY OF SOME FRESH VEGETABLES

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SUMMARY

Fresh vegetables are prone to be contaminated by foodborne pathogens during growth (fertilization or irrigation with wastewater), harvest, transport and further processing and handling. As most of these products are generally eaten raw or mildly treated, there is an increase in the number of outbreaks caused by bacteria associated with fresh vegetables. Fresh vegetables were analysed for aerobic plate counts, Escherichia coli, Salmonella sp., Listeria monocytogenes, Bacillus cereus, Staphylococcus aureus, Clostridium perfringens, Vibrio cholerae, Shigella sp., Campylobacter jejuni and mould counts. Based on the results, the microbial contamination of the studied vegetables was different, in some cases significant contamination with pathogenic bacteria was detected.

1. INTRODUCTION

The number of diseases transmitted by vegetables consumed raw show an increasing tendency (Sánchez et al., 2012). In contrast, the effective decree concerning the level of microbiological contamination of food include regulations and limit values only for *Salmonella*, *Escherichia coli* and molds (Regulation EüM 4/1998, Comission Regulation (EC) No 1441/2007). It is important to reveal the main contaminating sources and the real critical points (Lehto et al., 2011). The cause of the epidemics is mostly an enteral pathogenic bacteria which could reproduce before consumption or has low infective dose. Bacteria isolated from fresh vegetables: *Aeromonas hydrophila*, *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium botulinum*, *C. perfringens*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica* serotypes, *Shigella* sp., *Staphylococcus aureus*, *Vibrio cholerae* (McMahon and Wilson, 2001; Beuchat, 2002; Aycicek et al., 2005; Nguz et al., 2005; Abadias et al., 2008; Ponniah et al., 2010; Verhoeff-Bakkenes et al., 2011; Caleb et al., 2013). Raw vegetables produced without using any animal fertilizer, with the exeption of naturally occuring soil bacteria, do not contain pathogenic germs. In case of fertilization or irrigation with human or animal origin wastewater on the surface of the vegetables *Salmonella* serotypes, *Shigella* or *E. coli* can frequently be isolated. If wastewater or fertilizers are properly treated (e.g composting) the pathogenic microorganisms are inactivated or the number of the germs are singnificantly reduced. *Listeria monocytogenes* can be found in the soil or in vegetation and has long viability. It is capable of reproduction under refrigerated conditions too, and the modified atmosphere storage does not affect the reproductive rate, so that raw vegetables may be the source of human listeriosis. From the spore-forming bacteria *Bacillus cereus* and the soil-derived *Clostridium botulinum* can also be found, which through their spores represent an important source of infection for minimally processed products prepared for consumption (Valero et al., 2002; Deák, 2006, Sapers et al., 2006; Warriner et al., 2009; Leff and Fierer, 2013). Vegetables are excellent substrates for molds (including mycotoxin productive too), the most occurent are the microscopic filamentous fungi belonging to *Penicillium*, *Alternaria*, *Fusarium*, *Phoma*, *Cladosporium* genus (Tournas, 2005; Deák, 2006). Human pathogenic bacteria (e.g. *Campylobacter jejuni*, *Escherichia coli* O157:H7 and *Salmonella*) can survive longer in the rhizosphere than on the surface of the leaves. Besides endophytic bacteria populations bacteria belonging to *Salmonella*, *Staphylococcus*, *Mycobacterium*, *Klebsiella* genus could also be detected, which confirms the assumption that the pathogenic microbes can also penetrate inside healthy plant tissue (Sapers et al., 2006; Warriner et al., 2009).

2. MATERIALS AND METHODS

In the course of our work some vegetables (lettuce, tomato, pepper, cucumber, cabbage) from different commercial units and vegetable markets were microbiologically studied with cultivation methods. Some of the bacteria developed on each of the selective media with specific colony and occurring in higher number were identified using 16S rDNA gene sequence analysis. Sampling took place three times and the used vegetables were randomly chosen.

2.1. Detection of contaminating microorganisms with microbiological cultivation methods

During the examination of contaminating microorganisms we determined microorganisms defined in regulations (Regulation EüM 4/1998, Comission Regulation (EC) No 1441/2007): *Salmonella*, *Escherichia coli*, molds and other bacteria that do not appear in regulation but cause different diseases through food consumption: *Listeria monocytogenes, Bacillus cereus*, *Staphylococcus aureus, Clostridium perfringens, Vibrio cholerae*, *Shigella* sp., *Campylobacter jejuni*. We also determined the total plate count of mesophilic aerobic bacteria using standard nutrient agar medium. In the case of each bacteria the following selective media were used: *Salmonella* - Brilliantgrün Lactose Saccharose agar (Roth), *Escherichia coli* - TBX Chromo agar (Roth), *Listeria monocytogenes* - Listeria mono Diferential Agar Base (Fluka 77408), *Bacillus cereus* - Cereus Selective Agar (Fluka 22310), *Staphylococcus aureus* - Mannitol Kochsaltz Agar (Roth), *Clostridium perfringens* - Perfringens Agar Base (Fluka 39727), Clostridial differential broth (Fluka 27544), *Vibrio cholerae* - TCBS agar (Merck), *Shigella* sp. - XLD agar (Roth), *Campylobacter jejuni* - Campylobacter Blood-Free Selective Agar Base (modified CCDA, Merck). Occasionally specified supplementaries were added to the selective media. In case of microscopic filamentous fungi the samples were inoculated on Czapek-Dox media (Fluka 70185).

2.2. Identification of the selected bacteria using 16S rDNA gene sequence analysis

Identification at the species level of the selected bacterial isolates was realized using 16S rDNA gene sequence analysis. Bacterial isolates were cultivated in Nutrient Broth for 24 h at 28ºC. The genomic DNA was isolated from the 24 h liquid bacterial cultures using AccuPrep® Genomic DNA Isolation Kit, according to the manufacturer's protocol, the genomic DNA-s were detected in agarose gel. A part of the bacterial 16S rDNA gene was amplified with the universal oligonucleotides 27f 5' AGAGTTTGATCMTGGCTCAG 3' and 1492r 5'TACGGYTACCTTGTTACGACTT3' primers. The PCR products were checked out on 1% gel electrophoresis stained with Red Safe, using 1% TAE buffer. The amplification products were purified using a PCR purification kit (Fermentas GeneJET™ PCR Purification Kit) and the 16S rDNA fragments sequencing was performed with the help of sequencing services.

The obtained partial 16S rDNA gene sequences were edited and aligned with Chromas, phylogenetic analyses were conducted using MEGA 4 system. The bacterial strains were identified with the comparison of the partial 16S rDNA gene sequences found in the NCBI (National Center for Biotechnology Information) database using BLAST software. The bacterial strains identification was given based on the sequence similarities percentages.

3. RESULTS

Among the studied vegetables, in case of the lettuce and cucumber the contamination was higher compared to the other vegetables (tomato, pepper, cabbage) (Table 1., Table 2., Table 3., Table 4.). *Clostridium perfringens* was detected in one case, at the first sampling from lettuce as well as *Shigella* bacteria evidenced from the same sample. *Bacillus cereus* was not developed on the selective media in neither of the samplings. On the XLD agar in some cases yellow colonies have developed, typical of coliforms. The highest germ number could be observed at the second and fourth sampling in case of the lettuce ($5 \cdot 10^3$ CFU g^{-1}) and the cucumber ($2 \cdot 10^3$ CFU g^{-1}). Molds were isolated from 6 samples.

Table 1: The results of the microbiological study of fresh vegetables at the first sampling

Studied microorganisms (CFUg^{-1} sample)	Tomato	Pepper	Cucumber	Cabbage	Lettuce
Mesophilic aerobic bacteria	$1 \cdot 10^3$	$3 \cdot 10^2$	$1 \cdot 10^5$	$1 \cdot 10^4$	$2.08 \cdot 10^4$
Salmonella	0	0	$1 \cdot 10^4$	$1 \cdot 10^3$	$1 \cdot 10^4$
Escherichia coli	0	0	$2 \cdot 10$	0	$5 \cdot 10$
Shigella	0	0	0	0	$1.6 \cdot 10^2$
Campylobacter jejuni	0	0	$3 \cdot 10$	$1 \cdot 10^3$	$5 \cdot 10^3$
Vibrio cholerae	0	0	0	0	0
Staphylococcus aureus	10	0	0	0	$5 \cdot 10$
Listeria monocytogenes	0	$5 \cdot 10$	$9.7 \cdot 10^3$	$8.6 \cdot 10^2$	$1.61 \cdot 10^3$
Molds	$3.9 \cdot 10^2$	$6 \cdot 10$	10	0	0

Table 2: The results of the microbiological study of fresh vegetables at the second sampling

Studied microorganisms (CFU g^{-1}sample)	Tomato	Pepper	Cucumber	Cabbage	Lettuce
Mesophilic aerobic bacteria	$1.7 \cdot 10^2$	$4.1 \cdot 10^2$	$1.84 \cdot 10^3$	$3.72 \cdot 10^4$	$5 \cdot 10^5$
Salmonella	0	$2.1 \cdot 10^2$	$1 \cdot 10^3$	$1 \cdot 10^3$	$1 \cdot 10^3$
Escherichia coli	0	0	10	0	$1 \cdot 10$
Shigella	0	0	0	0	0
Campylobacter jejuni	0	$1 \cdot 10^3$	$1 \cdot 10^4$	$1 \cdot 10^4$	$1 \cdot 10^4$
Vibrio cholerae	0	0	0	0	$9.6 \cdot 10^2$
Staphylococcus aureus	10	$3 \cdot 10^2$	$2.6 \cdot 10^2$	$3 \cdot 10$	$4 \cdot 10^2$
Listeria monocytogenes	10	10	$1 \cdot 10^4$	$2.07 \cdot 10^3$	$5 \cdot 10^3$
Molds	0	0	0	0	$1 \cdot 10$

Table 3: The results of the microbiological study of fresh vegetables at the third sampling

Studied microorganisms (CFU g^{-1} sample)	Tomato	Pepper	Cucumber	Cabbage	Lettuce
Mesophilic aerobic bacteria	$2 \cdot 10^2$	$4.7 \cdot 10^2$	$5 \cdot 10^5$	$2.8 \cdot 10^4$	$1.08 \cdot 10^3$
Salmonella	0	0	$5 \cdot 10^3$	$5 \cdot 10^3$	$1 \cdot 10^4$
Escherichia coli	0	0	0	0	0
Shigella	0	0	0	0	0
Campylobacter jejuni	0	$1 \cdot 10^3$	$1 \cdot 10^3$	$1 \cdot 10^4$	$1.2 \cdot 10^2$
Vibrio cholerae	0	0	0	0	0
Staphylococcus aureus	0	$9.1 \cdot 10^2$	$8 \cdot 10$	$3 \cdot 10$	$4 \cdot 10$
Listeria monocytogenes	0	$1.32 \cdot 10^3$	$1 \cdot 10^3$	$1.4 \cdot 10^2$	$5.9 \cdot 10^2$
Molds	0	0	$1 \cdot 10$	0	0

Table 4: The results of the microbiological study of fresh vegetables at the fourth sampling

Studied microorganisms (CFU g^{-1} sample)	Tomato	Pepper	Cucumber	Cabbage	Lettuce
Mesophilic aerobic bacteria	$8.8 \cdot 10^2$	$1.57 \cdot 10^2$	$1 \cdot 10^4$	$1.49 \cdot 10^3$	$1 \cdot 10^4$
Salmonella	0	0	0	0	$1 \cdot 10^3$
Escherichia coli	0	0	$3.1 \cdot 10^2$	0	$5.8 \cdot 10^2$
Shigella	0	0	0	0	0
Campylobacter jejuni	$2 \cdot 10$	0	$3 \cdot 10^3$	$5 \cdot 10^2$	$3 \cdot 10^3$
Vibrio cholerae	0	0	0	0	0
Staphylococcus aureus	0	0	$5 \cdot 10^2$	$2{,}6 \cdot 10^2$	0
Listeria monocytogenes	$1.3 \cdot 10^3$	$4 \cdot 10^2$	$5 \cdot 10^2$	$5 \cdot 10^2$	$5 \cdot 10^2$
Molds	$1 \cdot 10^2$	0	0	0	0

On the basis of 16S rDNA BLAST results the 15 bacterial isolates originated from the fresh vegetables belong to five different genus, showing 10 isolates 99% similarity with species described in GenBank. Six isolates were identified as belonging to genus *Enterobacter* (the species are *Enterobacter ludwigi* 99% from lettuce and *Enterobacter hormaechei* 99% from cucumber). One isolate from the cucumber sample has no significant similarity in NCBI, but in EzTaxon shows similarity with *Enterobacter pyrinus* 84.17%. Four bacterial strains (originated from lettuce, cucumber and cabbage samples) showed similarity with strains from *Acinetobacter* genus (*Acinetobacter calcoaceticus* 99% isolated from lettuce and cabbage). According to the 16S rDNA BLAST data one isolate from lettuce was identified as *Providencia rettgeri* and one from tomato as *Aeromonas hydrophila*, both with 99% similarity. Other three bacteria belong to *Bacillus* genus: *Bacillus methylotrophicus* 95% from lettuce, *Bacillus cereus* 99% and *Bacillus anthracis* 99% from cucumber.

4. CONCLUSIONS

Considering the regulations of the decree the studied vegetables were microbiologically objectionable in 12 of the cases. Beside the bacteria prescribed in regulations, in most of the cases other bacteria causing different diseases were detected, fact confirmed by the results of molecular biology methods, too.

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DISCRIMINATION OF BLACK TEAS BY GC-MS ANALYSIS, SENSORY PROFILE ANALYSIS AND ELECTRONIC NOSE AND TONGUE

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SUMMARY

The tea made from the Camellia sinensis plant's leaves is among the most consumed beverages. Nowadays its agents got to the centre of attention due to the discovery of the beneficial effects of regular tea consumption. Defining the quality of the tea is very hard, the classifications of the finished products are primarily done by sensory methods; the basis of that method is the classification of the various flavours in teas. The aim of our work was the complex analysis of the flavour of black teas. The GC-MS analyses were compared with electronic nose and tongue device tests and the results of the sensor profile analysis to decide whether the instrumental or the sensory examination are more suitable to analyse the tea's quality and characteristics.

1. INTRODUCTION

The tea made from the Camellia sinensis plant's leaves is among the most consumed beverages. Nowadays its agents got to the centre of attention due to the discovery of the beneficial effects of regular tea consumption.

Defining the quality of the tea is very hard; the classification process is partly objective, with instrumental methods, and partly subjective, with sensory methods (Owuor, 2005). The sensory characteristics are determined by the variety, the growing conditions and the processing method of the plant. To the present day the classification of the finished products are primarily done by sensory methods; the basis of that method is the classification of the various flavours in teas. The instrumented physical (colour and conductivity measurements) and the analytical measurements (gas chromatography, liquid chromatography and multi-element analysis) are often used to supplement and confirm the sensory method (Kim *et al*., 2007).

2. MATERIALS AND METHODS

2.1. Examined samples

The examined samples were provided by Sara Lee Hungary. The packaging and the distribution of teas occur in Hungary, the processing takes place at the location of the cultivation. Analyses were performed with the following samples: Chinese Keemun black tea, Ceylon black tea, Indian Assam black tea and Indian Darjeeling black tea.

2.1. Methods

The preparation of the samples plays a crucial role in completing a successful analysis. The modified Likens-Nickerson simultaneous distillation-extraction is the most effective method for extracting the components that are responsible for the fragrance (Zhu *et al*., 2008). The aroma compounds of teas were separated with gas chromatography, using a polar capillary column, the detection and identification of components was carried out with mass

selective detector (Hewlett Packard 5890 series II. GC + 5971/A MSD). The evaluation of the results was performed with the flavour spectrum method developed by the Department of Food Chemistry and Nutrition on the Corvinus University of Budapest.

The comparison of black teas was performed based on all constituents and aroma-spectra of the samples. In addition the results of GC-MS analysis were compared with sensor profile analysis (ISO 11035:1994), electronic nose (NST-3320) and electronic tongue (Alpha Astree II.) analysis.

3. RESULTS

The black teas belong to two subtypes: the Indian Assam and the Ceylon black tea are representatives of the *var. assamica* subtype, the Chinese Keemun and the Indian Darjeeling belong to the *var. sinesis* subtype. This classification made possible the comparison of genetically identical cultivated variants and the discovery that whether the different place of cultivation and the climatic conditions are affecting the formation of the flavour compounds. The Indian teas' similarity and richness in flavour become apparent from the spectrum, also the Darjeeling tea's superiority in the relative intensity of some components is revealed (Fig. 1).

The botanical identity of the types suggested that the Ceylon and Assam teas' spectral pattern, and the Darjeeling and Keemun tea's spectral pattern will be similar. However, more obvious similarity showed between the two above mentioned Indian teas and the Ceylon tea. The resemblance in the patterns appeared mainly in the first half of the flavour spectrum, among the 1000-1700 PTRI components. These components are from the tealeaf's precursors, and their quantities are genetically determined. Therefore the differences are in the flavours formed by the production technology. Based on the spectrum, the Chinese tea differs from the other black teas. The profile drawn from the sensory evaluation's rating, showing the samples'odour characteristics, is comparable with the flavour spectra. The Ceylon, the Assam and the Darjeeling teas' profile are very similar to each other, while the Keemun's profile is different from the others.

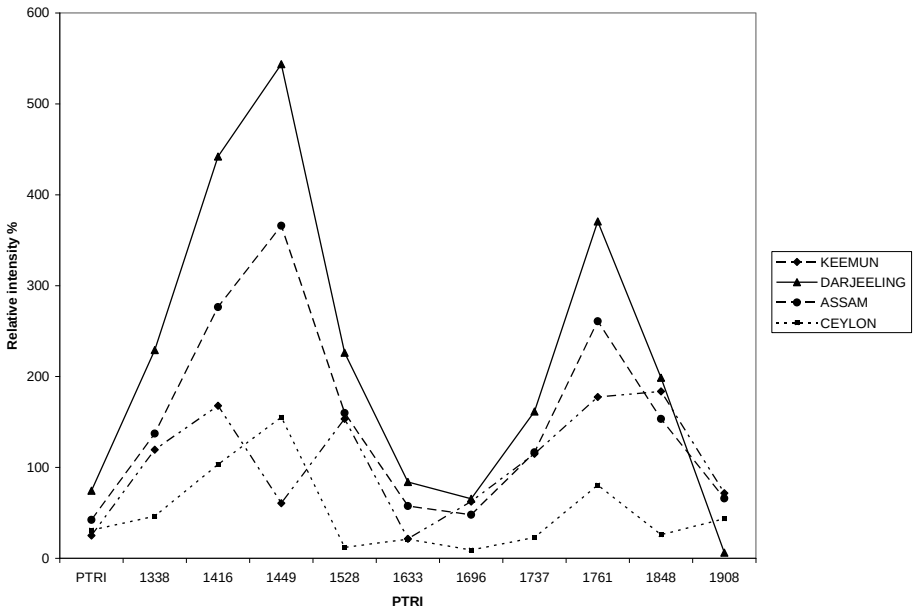

Figure 1: Aroma spectra of black teas

During the electronic nose tests the Indian Assam and Darjeeling teas similarity was proven. The Ceylon black tea related to the Indian Assam, both belong to the *var. assamica* cultivar. Their quality rating in the statistical assessment is close to each other. The discriminant analysis also shows that the separation of the Chinese corresponds with the flavour spectra, thus the obtained results are confirm the gas chromatography measurements (Fig.2.).

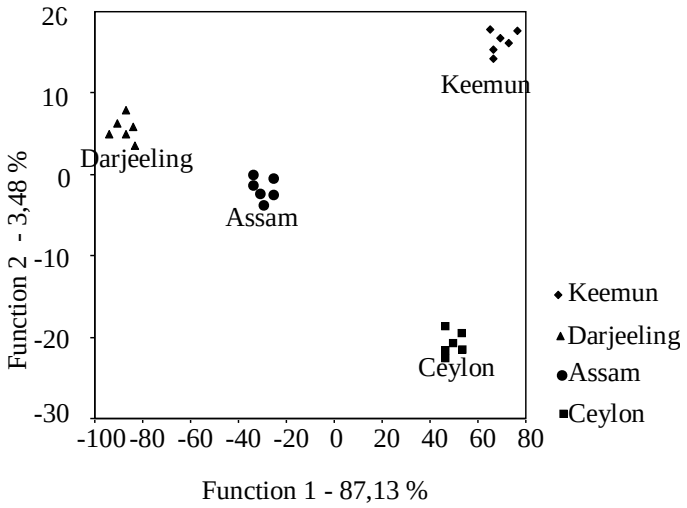

Figure 2: Discriminant plote of black teas by electronic nose analysis

Comparing the results of the tea leaf's and beverage's electronic nose device tests it was determined that the leaf's volatile components were more suitable for separation with this device. These components have the lowest boiling point and are the most volatile parts of the flavouring substance; therefore they can be extracted from the tea with head space sample preparation. Further test are needed to prove if these components are enough to show the difference between teas belonging to the same subtype or originating from the same region. In the tea beverage's case, the separation of the samples is less reliable based on fragrances gained from the vapour.

Table 1: Pairwise significant difference matrix

global odour	**sd(5%)= 16,50**		**sd(1%)= 22,13**	
	Ceylon	Darjeeling	Assam	Keemun
Ceylon	-	no	no	1%
Darjeeling	14,5	-	no	no
Assam	9,9	4,6	-	5%
Keemun	**28,5**	14	**18,6**	-
global taste	**sd(5%)=10,65**		**sd(1%)=14,27**	
	Ceylon	Darjeeling	Assam	Keemun
Ceylon	-	no	1%	1%
Darjeeling	4,8	-	1%	no
Assam	**20**	**15,2**	-	no
Keemun	**15**	10,2	5	-

The results of the sensory examination (Table 1) are corresponding well with the electronic tongue and nose device's results. Panellists found significant differences in global odour between the Ceylon and Keemun tea and the Assam and Keemun tea. There's a 99% difference in global taste characteristics between the Ceylon and Assam tea, the Ceylon and Keemun tea and the Darjeeling and Assam based on the ratings given by the reviewers (Fig.3.). The results of the electronic nose and tongue device show that the methods can be applicable for differentiating teas based on their tastes.

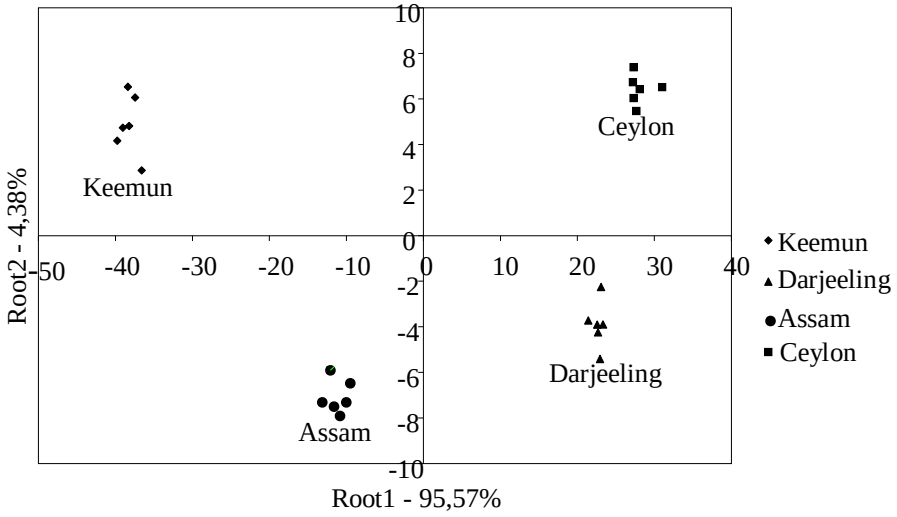

Fig.3: Discriminant plote of black teas by electronic tounge analysis

4. CONCLUSIONS

The analyses performed with electronic nose and tongue devices look promising; these make possible the fast inspection of teas: the electronic devices are able to detect differences between the black teas more reliably than the subjective sensory examination. The fast and simple sample preparation and the short-time examination allow this method to be used as an objective quality control in the trade to check the origin of the teas. If the electronic sensors detect any difference, the background of this divergence can be explored with GC-MS investigations, and justify the difference between the samples. This also can be confirmed with the marker compound characteristics of the cultivars, which were identified during my examinations as well.

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ISOLATION, IDENTIFICATION AND DIVERSITY OF FOOD SPOILAGE YARROWIA YEAST STRAINS FROM DIFFERENT FOODS

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SUMMARY

Yeasts of the Yarrowia clade frequently occur in meet and milk products. In many cases they cause spoilage due to their strong lipolytic and proteolytic activity, and their ability to produce brown pigments.
Using conventional methods most of these yeasts can be misidentified as Yarrowia lipolytica, but using molecular biological methods it can be stated, that they are members of a complex group including several different species.
Our aims were to find a proper method to collect Yarrowia strains, and to assign them to groups based on their physiological and molecular characteristics, then, following sequence-based identification of selected members of each group to determine the composition of the species.

1. INTRODUCTION

Yarrowia lipolytica is known as one of the most dangerous and one of the most common spoilage yeasts of the food industry (Deák, 2008). Yeasts of the *Yarrowia* clade occur mainly in meet and milk products and cause spoilage in many cases, due to their strong lipolytic and proteolytic activity, and their ability to produce brown pigments (Roostita & Fleet, 1996; Suzzi et al., 2001, Gardini et al., 2006). Using conventional methods most of these yeasts can be misidentified as *Yarrowia lipolytica*, but using molecular biological methods it can be stated, that they are members of a complex group including several different species (Péter et al., 2004; Kurtzman, 2005; Knutsen et al., 2007; Limtong et al., 2008; Groenewald & Smith, 2013) (Figure 1.).

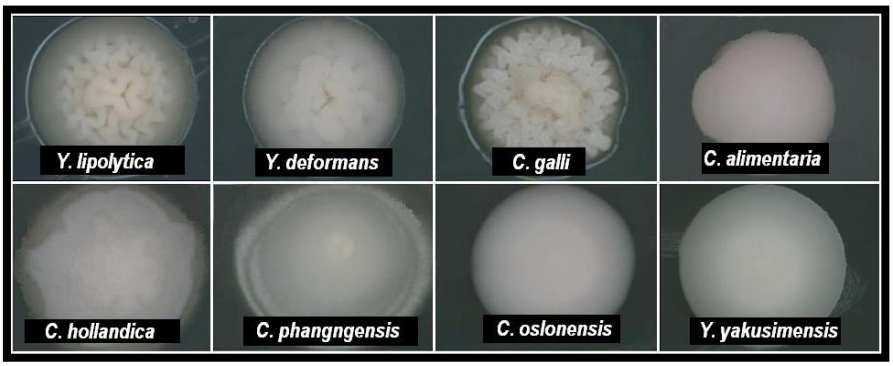

Figure 1. Colony morphology of the type-strains of the *Yarrowia* group

Our aims were to find a proper method to collect *Yarrowia* strains, and to assign them to groups based on their physiological and molecular characteristics, then, following sequence-based identification of selected members of each group to determine the composition of the species.

2. MATERIALS AND METHODS

For the selective isolation of yeast strains of *Yarrowia* clade, first we used a three-step enrichment in hexadecane-containing liquid medium, pH 3.6. After the enrichment serial decimal dilutions and surface plating on Rose-Bengal Chloramphenicol (RBC) agar were made. Following the incubation on 25°C for one week, strains representing different colony types were isolated and purified by repeated streaking on Glucose-Peptone-Yeast extract (GPY) agar. The ability of the isolated strains to grow on hexadecane was confirmed before further investigation, by inoculating strains into a medium containing hexadecane as a sole carbon source, and then incubated at 25°C for 3 weeks.

From the hexadecane positive strains the members of the *Yarrowia* clade were selected by additional physiological tests. Their glucose-fermentation, nitrate-assimilation, and the assimilation of 30 different carbon-sources by using API ID32 C tests were examined, following the manufacturer's recommendations.

Strains, selected based on their physiological characteristics were assigned to groups using microsatellite PCR. To determine the composition of the species, selected members of each group were identified by amplifying the D1/D2 region of the ribosomal RNA's large subunit coding gene (Kurtzman & Robnett, 1998), then comparing the sequences to sequences found in the GenBank database using BLAST 2.2.28 program (Zhang *et al.*, 2000).

3. RESULTS AND DISCUSSION

645 yeast strains were isolated from 118 samples of different foods: raw meet (pork, beef and poultry), raw milk and expired milk products (cheese and cottage cheese). 260 of them can assimilate hexadecane (Figure 2.), from which 164 strains were assigned as members of the *Yarrowia* clade (Figure 3.) by examining their above-mentioned physiological parameters.

Using molecular biological methods strains from raw meet were assigned to seven species of the *Yarrowia* clade. Four of them are described species, but three of them are new ones. Earlier, based on conventional phenotypic tests, all of these strains would have been identified as *Y. lipolytica*, though actually only 20% of them belong to this species, 35% of them belong to *Yarrowia deformans*, 15% of them to *Candida galli*, 10% of them to *Candida alimentaria*, 20% of them can be assigned to three new species (Figure 4.).

Yarrowia strains from raw milk and milk products also will be further examined and identified at species level.

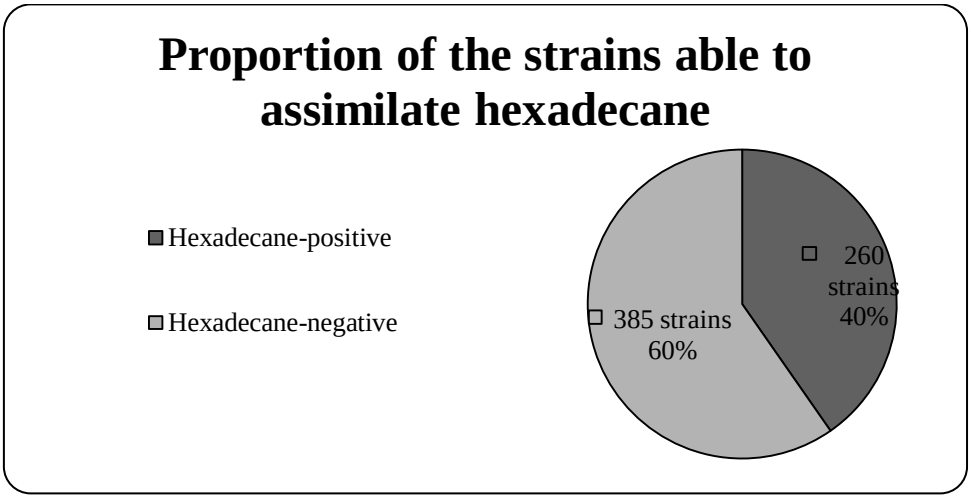

Figure 2. Hexadecane assimilation of the isolated strains

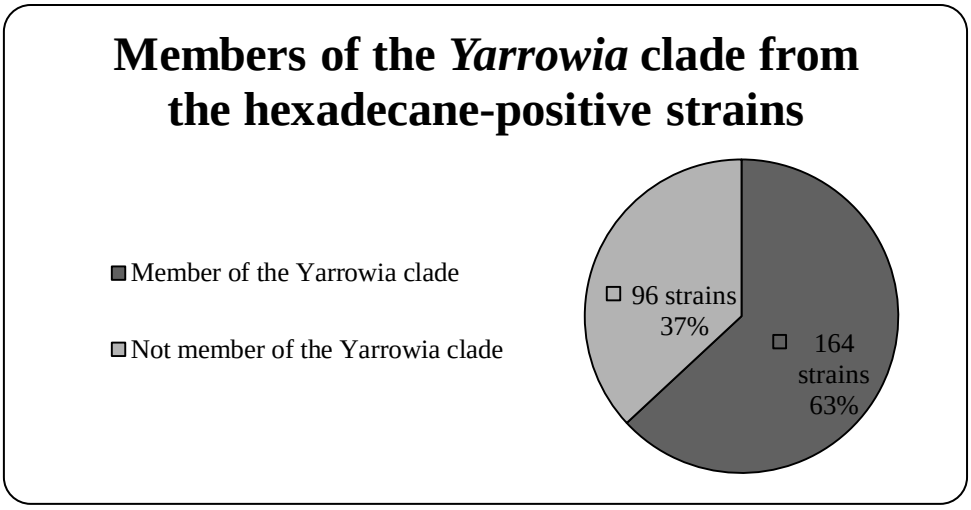

Figure 3. Members of the *Yarrowia* clade from the hexadecane-positive strains

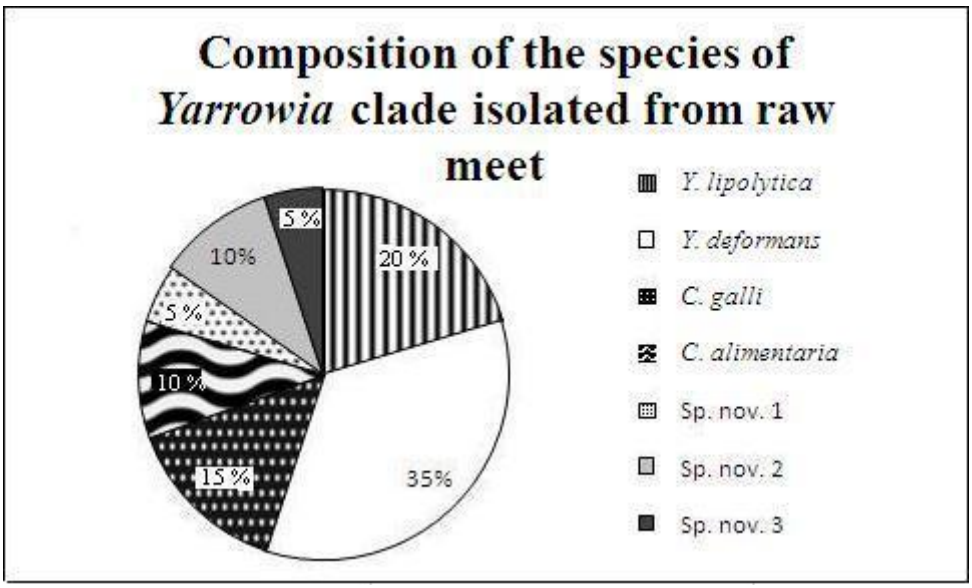

Figure 4. Composition of the species of *Yarrowia* clade isolated from raw meet

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CHARACTERIZATION OF HUNGARIAN HONEYS FROM CSONGRÁD COUNTY IN TERMS OF MINERAL CONTENT

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SUMMARY
Honey is a key product of traditional agriculture in the southern region of the Great Hungarian Plain and it is also considered an important export item in Hungarian commerce. Our aim in this study was to determine the macro- and microelement content in samples from Csongrád County and compare them in terms of variety and origin within the county. The measurements were carried out using ICP-MS. Our results show that the element found in highest amount in samples collected in the region was potassium and sodium. As for microelements; boron, zinc, iron and copper were present in the largest amounts.

1. INTRODUCTION

The quality of food has a great economic importance for the sectors involved in food production, processing or packing and also for the consumers. Quality is therefore also crucial in honey production, a developing agricultural sector in Hungary. Due to geographical and climatic conditions, honey production and beekeeping have been important activities for hundreds of years in Hungary and in the Great Plain (Szűcs, 2010). According to the data provided by the National Hungarian Apiculture Association (Országos Magyar Méhészeti Egyesület, OMME), there were 18,782 beekeepers in Hungary in 2011 with 1,065,860 colonies of bees producing about 20,000 tons of honey (Tóth, 2011). However, there are some well-defined regions in Hungary, such as the Southern Great Plain, which provides the opportunity for quality food and honey production in Hungary. The Hungarian Great Plain is a mosaic of steep sand dunes and depressions characterized by a continental climate and poor soil conditions (Kovács and Szigetvári, 2002). These environmental conditions have created a special endogenous plant community and unique possibilities for agriculture. The geographical origin and the special plant sources determine the characteristics and the quality of honey produced in Csongrád County, the southern region of the Hungarian Great Plain. Many types of unifloral (robinia, sunflower, rapeseed, milkweed) and multifloral honeys are produced by about 28,000 honey bee colonies in this region (Tóth, 2011).

Mineral content is one of the most important attribute of honey which depends on the geographical origin of honey and thus can be used as environmental indicator for the classification of honeys (Anklam, 1998; Hernandez et al., 2005; Bogdanov et al., 2007; Tuzen et al., 2007; Kaškonienė and Venskutonis, 2010; de Alda-Garcilope, 2012). The macro-(K, Ca, Mg, Na) and microelements (B, Al, Mn, Fe, Co, Ni, Cu, Zn, Se, Mo) from the soil, are transported to the plant nectar which is transformed into honey by a process of regurgitation and evaporation by honey bees. The element composition of honey types varies from soil to soil and also in plant species (Przybylowski and Wilczyńska, 2001; Stankovska et al., 2008).

The mineral content of Hungarian honeys have been studied by a small number of researchers (Kasper-Szél et al. 2003; Zajácz et al., 2008; Czipa et al., 2008) and no data are available on the macro- and microelement contents of southern Hungarian honeys. Our aim was to study the mineral contents of unifloral honeys from Csongrád County and to compare the macro- and microelement contents of honey samples from this region.

2. MATERIALS AND METHODS

2.1. Samples

Honey samples were collected from different locations in Csongrád County, Hungary in 2013. The honey samples were obtained directly from beekeepers. All samples were unpasteurized and were collected no more than three months before analysis. Samples were stored in tubes and kept at room temperature in the dark. The declared botanical origin by the producers was considered and one sample was from each sampling point.

2.2. Determination of mineral content of honey sample

Concentrations of macro- and microelements were determined using an XSeries II ICP-MS instrument (Thermo Scientific, Bremen, Germany). 100 mg honey was homogenized and placed in test tubes containing 6 ml concentrated nitric acid and 2 ml H_2O_2 for 2 h. The samples were digested in a microwave destructor (MarsXpress CEM, Matthews NC, USA) at 200^{o}C for 15 min. Samples were then cooled, diluted with 12 ml double distilled water. Macro- and microelement contents are expressed in ppm or ppb. Data presented as average values ±SD, n=3.

3. RESULTS

Comparison of the macro- and microelements in the different unifloral honeys revealed differences (table 1.). The concentration of the elements appeared to depend not only on the geographical origin of the honeys, but also on the botanical source. The highest content of K (603.1 ppm), Ca (140.8 ppm) and Mg (34.09 ppm) were found in the sunflower honeys. The range of Na was 170-301 ppm in the honeys. The maximum Fe levels (8.36-13.47 ppm) as well as the highest B (15.83 ppm), Mn (275 ppb) and Mo (74.19) concentrations were observed in the rapeseed honeys. The highest concentration of selenium (100.8 ppb) was in the milkweed honeys. Concentration of microelements ranged from 1.19 to 2.81 ppm for copper, 2.99-8.89 ppm for zinc and 89.34-153.6 ppb for nickel in the honey samples from Csongrád County. The lowest microelement level was that of Co in the honeys samples (0.72-22.05 ppb).

Table 1: Macro- and microelement contents of unifloral honey from Csongrád County

Elements	Studied areas											
	Robinia			Rapeseed			Sunflower			Milkweed		
	Mórahalom	Röszke	Üllés	Baks	Kiszombor	Röszke	Hódmező-vásárhely	Maroslele	Szentes	Csongrád	Balástya	Öttömös
K (ppm)	183.7 ± 0.69	188.5 ± 1.57	385.6 ± 2.38	202.8 ± 1.92	439.8 ± 1.89	244.4 ± 1.25	465.9 ± 0.90	603.1 ± 2.49	460.3 ± 1.88	328.3 ± 1.9	198.5 ± 1.29	235.3 ± 0.99
Ca (ppm)	23.08 ± 0.13	21.6 ± 0.21	22.36 ± 0.13	24.55 ± 0.62	38.3 ± 0.15	141.9 ± 0.96	109.3 ± 0.47	103.8 ± 0.70	140.8 ± 0.66	18.46 ± 0.04	25.95 ± 0.18	17.07 ± 0.13
Mg (ppm)	1.53 ± 0.09	1.03 ± 0.12	7.60 ± 0.66	7.21 ± 0.16	22.77 ± 0.18	17.4 ± 0.14	27.33 ± 0.13	34.09 ± 0.36	24.61 ± 0.19	5.77 ± 0.03	4.44 ± 0.06	0.65 ± 0.144
Na (ppm)	179.4 ± 0.85	170.5 ± 2.69	226.3 ± 1.86	268.9 ± 4.09	192.8 ± 0.86	257.4 ± 1.34	202.5 ± 2.63	255.5 ± 1.72	301.2 ± 0.77	219.4 ± 2.03	174.9 ± 2.98	225.8 ± 1.30
Fe (ppm)	6.68 ± 0.01	4.95 ± 0.04	4.52 ± 0.03	8.36 ± 0.26	8.63 ± 0.06	13.47 ± 0.21	5.67 ± 0.22	7.10 ± 0.14	8.12 ± 0.25	5.70 ± 0.31	5.23 ± 0.16	6.01 ± 0.06
Cu (ppm)	1.19 ± 0.26	1.80 ± 0.14	2.31 ± 0.17	2.04 ± 0.22	1.82 ± 0.28	2.06 ± 0.29	2.29 ± 0.23	2.74 ± 0.16	2.09 ± 0.09	2.15 ± 0.19	1.56 ± 0.11	2.81 ± 0.02
Zn (ppm)	6.44 ± 0.12	6.21 ± 0.08	4.50 ± 0.02	7.11 ± 0.11	2.99 ± 0.10	8.49 ± 0.01	4.99 ± 0.10	7.21 ± 0.10	8.89 ± 0.05	6.63 ± 0.09	7.49 ± 0.03	8.58 ± 0.09
B (ppm)	5.19 ± 0.08	4.52 ± 0.06	8.83 ± 0.51	5.06 ± 0.11	12.85 ± 0.22	15.83 ± 0.23	9.04 ± 0.29	10.02 ± 0.52	9.14 ± 0.29	5.47 ± 0.14	4.76 ± 0.18	6.65 ± 0.18
Mn (ppb)	152.9 ± 2.92	101.4 ± 2.45	178.0 ± 3.57	56.15 ± 3.33	249.8 ± 0.46	275.0 ± 2.40	167.8 ± 2.10	183.1 ± 1.87	164.1 ± 5.38	120.2 ± 2.60	104.0 ± 1.37	95.33 ± 2.69
Ni (ppb)	94.45 ± 4.19	99.21 ± 5.51	108.1 ± 2.58	93.56 ± 8.98	89.34 ± 3.82	97.43 ± 6.74	97.44 ± 4.92	153.6 ± 1.80	126.8 ± 4.82	104.1 ± 9.39	96.95 ± 20.82	100.7 ± 5.76
Se (ppb)	72.57 ± 6.39	58.67 ± 13.01	84.64 ± 11.5	9.48 ± 29.2	67.73 ± 6.28	89.05 ± 10.42	71.17 ± 19.23	77.14 ± 10.86	35.16 ± 30.77	82.04 ± 16.62	85.9 ± 4.77	100.8 ± 4.51
Mo (ppb)	30.25 ± 3.77	21.46 ± 0.91	9.79 ± 6.18	3.97 ± 0.85	7.77 ± 1.73	74.19 ± 0.97	15.29 ± 2.86	9.52 ± 2.28	12.22 ± 3.45	12.66 ± 2.39	6.3 ± 2.56	9.42 ± 1.23
Co (ppb)	4.37 ± 0.25	2.28 ± 0.33	11.77 ± 0.15	0.72 ± 0.18	9.18 ± 0.61	22.05 ± 1.06	3.24 ± 0.37	3.64 ± 0.55	2.95 ± 0.69	2.63 ± 0.38	2.09 ± 0.48	2.39 ± 0.29

4. DISCUSSION

In conclusion, the element found in highest amount in the honey samples collected in Csongrád County was potassium, followed by sodium. The potassium, calcium and magnesium content showed the most characteristic differences between the honeys (sunflower>rapeseed>robinia=milkweed). The highest contents of B, Mn and Mo concentrations were observed in the rapeseed honeys. The highest concentration of selenium was detected in the milkweed honeys. The non-essential plant microelement found in the lowest level was Co.

In addition, not only the botanical source but also the geographical origin determined the macro- and microelement contents in the honeys.

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COMPARATIVE ANALYTICAL STUDIES ON BIOACTIVE COMPONENTS OF VARIOUS HUNGARIAN PROPOLIS SAMPLES WITH SPECIAL REGARD TO THEIR FUNCTIONAL FOODSTUFF RELATED APPLICATIONS

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SUMMARY
Propolis is a resinous mixture collected by honey bees from tree buds, sap flows, or other botanical sources. The composition of propolis depends on time, vegetation, and the area of collection. This study examined the antioxidant, antiradical and antibacterial activities of ethanolic extract of propolis (EEP) and compared total levels of polyphenolic compounds, prolin, vitamins and microelements. Samples were collected from different geographical regions of Hungary. The major constituents of ethanol extract of propolis were analyzed by high-performance liquid chromatography (HPLC) analysis, and the total phenolic compounds were characterized using the Folin–Ciocalteu reagent. The free radical scavenging activities of propolis samples were evaluated by using the DPPH assay. The main flavonoid componets were pinocembrin, chrysin, apigenin and vanillin. The in vitro inhibitory activity of propolis extracts against eight bacterium strains was investigated by the agar well diffusion assay.

1. INTRODUCTION

Propolis is a strongly adhesive, resinous substance collected, transformed and used by bees to seal holes in their honeycombs, smooth out the internal walls and protect the entrance against intruders. Honeybees (*Apis mellifera* L.) collect the resin from the cracks in the bark of trees and leaf buds. This resin is masticated, salivary enzymes added and the partially digested material is mixed with beeswax and used in the hive. The precise composition of raw propolis varies with the source. In general, it is composed of 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen and 5% various other substances, including organic debris (Budock et al., 1998)

Flavonoids and various phenolic compounds are the most important pharmacologically active constituents in propolis and have been shown to be capable of scavenging free radicals, thereby protecting lipids and other compounds (such as vitamin C) from being oxidized or destroyed during oxidative damage (Hegazi and Abd El Hady, 2002). Propolis has also gained popularity and is used extensively in drinks and foods to improve health and prevent diseases such as inflammation, heart disease, diabetes and even cancer (Mishima et al., 2005). Because of these properties and the different uses of propolis, there is a renewed interest in its anti-oxidant activities. Several investigations in Europe and South America have indicated that flavonoids extracted from propolis are powerful anti-oxidants (Kumazawa et al., 2004) that can protect serum lipoproteins from oxidation (Isla et al., 2001; Sulaiman et al. 2011). Different propolis chemical profiles are associated with the presence of specific compound types, such as polyphenols, terpenoids, prenylated acetophenones, isoflavonoid. Their contents could be responsible for the biological activities of propolis varieties; therefore, the study of samples from areas where propolis has never been studied before could reveal new propolis types and new propolis constituents of important biological activity (Popova et al. 2011).

As a result of the wide biological activities, propolis is extensively used in the food (beverages, health foods, and nutritional supplements, candy) and cosmetics industry (toothpaste, syrup, soap) and medicine as well as in home remedies. Furthermore, propolis can also cause some serious side effects such as allergic reactions in some individuals (Münstedt 2009).

In the present study, we are reporting the chemical composition, antioxidant activity and the antimicrobial properties of propolis samples collected from Hungary.

2. MATERIALS AND METHODS

2.1. Propolis

The samples were obtained by beekeepers from six regions of Hungary: PRO around Noszvaj (Heves County); PBA, DAC, PEP around Mezőkövesd (Borsod-Abaúj-Zemplén County); PHA around Debrecen (Hajdú-Bihar County); PDU around Pusztaszabolcs (Fejér County) in 2008 Spring-Summer. Propolis samples were collected from directly the hive and the samples were kept under dry and dark conditions before the processing and analysis.

2.2. Preparation of ethanolic extracts of propolis (EEP)

Propolis samples were frozen at -20 °C, and ground in a chilled mortar. Then, 200 g ground propolis powder was extracted with 1000 ml ethanol:water (80:20 / v:v) with continuous stirring at room temperature for 24 h. The EEPs were kept at room temperature under dark conditions.

2.3. Total polyphenol determination

Total polyphenol contents in extracts were determined by the Folin–Ciocalteau colorimetric method. EEP (0.5 ml) was mixed with 0.5 ml of the Folin–Ciocalteau reagent and 0.5 ml of 100 mg/ml Na_2CO_3, and the UV/VIS absorbance was measured at 760 nm after 1 h of incubation at room temperature. The calibration curve was obtained using 1 – 100 ppm gallic acid solutions prepared with water. Total polyphenol contents were expressed as mg/g (gallic acid equivalents) (Waterhouse, 2001).

2.4. Free radical-scavenging activity (DPPH)

1.5 ml of diluted propolis extracts (10–100 mg/ml) were added to 0.5 ml of 300 mmol/l DPPH in ethanol. The mixtures were shaken vigorously and left to stand at room temperature for 20 min in the dark. Absorbance at 517 nm was measured using ethanol as a blank. The degradation of DPPH was evaluated by comparison with a control (0.5 ml of DPPH solution and 1.5 ml of ethanol). Results were expressed by the proportion of DPPH degradation compared with the control.

2.5. Antimicrobial tests

One mL of inoculum (fresh cultures of bacterial strains: *Bacillus subtilis* and *Pseudomonas aeruginosa*) was mixed with 22 mL of MüllerHinton agar. After cooling, the inoculated plates were incubated at room temperature for 25 min, then holes (d = 6 mm) were made with stainless steel cylinders. Four holes were made per plate. 40 µL of EEP was dropped into the holes and 40 µL of 80 % ethanol as control. In order to accelerate the diffusion of EEP into the agar, the plates were incubated at 4 °C for 1 h and then at 37 °C for 18 h under aerobic conditions. The diameter of the inhibition zone around each hole was measured and recorded.

2.6. Microelement determination

Iron and zinc contents of the samples were measured with Flame Atomic Absorption Spectrometry (Varian Spectra) at a wavelength of 248.3 nm (iron) and 213.9 nm (zinc).

2.7. Prolin determination

Proline was determined spectrophotometrically by using ninhydrin in methyl cellosolve, and absorbance was read at 512 nm. A standard curve was made by using pure proline.

2.8. Niacin determination

Niacin was determined by the HPLC-PDA apparatus. The eluent was methanol:TFA (0,005 %). The flow rate was 0.8 ml/min, detection was performed at 194 nm and 260 nm. The column was Agilent (Zorbax, Eclipse Plus C18 4.6x250 mm; 5 µm).

3. RESULTS

3.1. Antioxidant activity and total polyphenol content

In the PRO, DAC and PDU samples the antioxidant activity was found to be below 1,000 ppm, whereas it was above 1,600 ppm in the other samples. Similarly to this, the values of DAC and PDU were lower than that of the other samples. The PBA sample contained the highest amount of polyphenols and antioxidants (1789.5 and 261.9 ppm) (Fig.1).

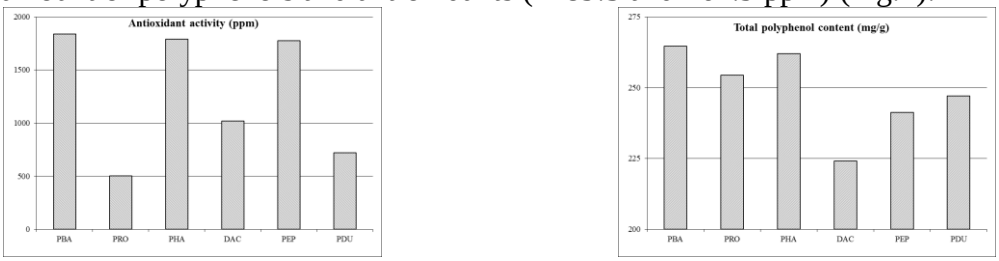

Figure 1: Antioxidant activity and Total polyphenol content of the samples

3.3. Iron and zinc content

Iron content of the samples was nearly identical (0.46 - 1.22 ppm) but the PBA contained 2.78 ppm iron, which is a significantly higher amount than those found in the other samples. The PRO, DAC, PEP and PDU samples contained 1.22 - 1.57 ppm zinc, but the PBA and PHA samples contained only 0.34 - 0.47 ppm zinc (Fig.2).

Figure 2: Zn content and Fe content of the samples

3.4. Proline and niacin content

Proline and niacin are representative components of the propolis. The proline was found about in the same amount in the samples (approx. 200 ppm), whereas the niacin showed higher deviation: 0.007 mg/ml both in the PPA and PHA samples and 0.001 - 0.002 mg/ml in the other samples (Fig.3).

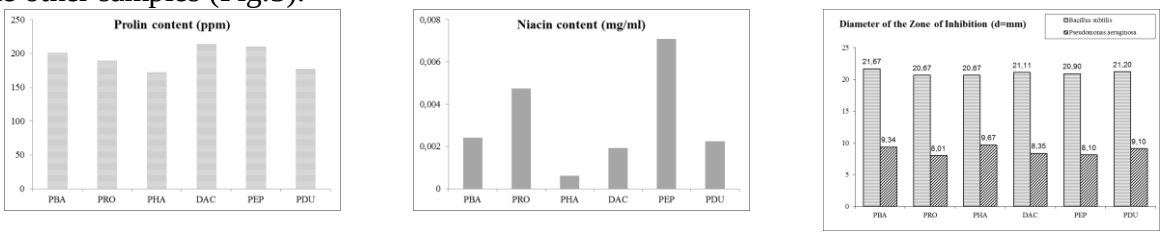

Figure 3: Prolin content, Niacin content and Diameter of the Zone of Inhibition

3.5. Microbiological results

In the course of the microbiological investigations the antimicrobial effect of the various propolis samples was confirmed both for Gram positive and Gram negative bacteria. Differences in the inhibitory effect were not found between the samples but between the bacterium species. The inhibition zone was twice as big for the *Bacillus subtilis* as for the *Pseudomonas aeruginosa* (Fig.4).

4. SUMMARY

The propolis is a very complex substance, consisting of many components. Many important differences were found in the course of the investigations, which can be related to the different plant origin. The bees always use the most favourable source in the given area, so quite large differences can be found in the composition of samples collected at the same time but in different areas or collected in the same region but in different periods. Nevertheless, many parameters are very similar in honeys collected from various sources, e.g. proline content or antimicrobial effect. These parameters are very important in respects of the bees' physiology.

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GLYCOSYL TRANSFERASE AND REVERS HYDROLYTIC ACTIVITY OF PECTINEX ULTRA SP-L ON DIFFERENT SUBSTRATES

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SUMMARY
Pectinex ultra SP-L commercial enzyme preparation has already used for synthesis of different carbohydrates (oligosaccharides) mainly fructooligosaccharides or galactooligosaccharides. This enzyme preparation exhibited hydrolase on trehalose substrate and was able to catalyse glucosyl transfer reaction on turanose, palatinose, maltulose, and cellobiose substrates and galactosyl transferase reaction on melibiose producing oligosaccharides. The highest glucosyltransfer activity was detected on palatinose substrate. Ín the case of 30 g/100ml palatinose sustrate concentration, 26 µl/ml enzyme, at pH5.5, 60 °C, 2.06 g/100ml oligosaccharide was synthetised on the second day of bionconversion. Pectinex also catalyse reverse hydrolysis reaction on glucose and mannose monosaccharide substrates. On mannose substrate disaccharides were produced and on glucose disaccharides and oligosaccharides as well. These results are very promising todevelop a new technology for biosynthesis of certain carbohydrates.

1. INTRODUCTION

Carbohydrates play an important role in biological systems such as energy source, cell-cell communication, prebiotics etc. The enzyme-catalyzed synthesis of carbohydrates represents an interesting alternative allowing the control of both the regioselectivity and the stereochemistry of bond formation, wide range of reaction conditions and the possibility of immobilization. Two main types of enzymes are used to catalyze oligosaccharide synthesis: hydrolases (glycosidases: EC 3.2) and transferases (glycosyl-transferases (glycosyl-transferases: EC 2.4)(Perugino *et al.*, 2004, Maitin *et al.*, 2007). Several oligosaccharides (OS) with high industrial interests are already marketed. However, in the last some years, the interest of new types ofcarbohydrates with specific sizes and containing specific glycopyranosyl-residueshas become to front (Homann & Seibel, 2009).

In this study, glycosyltransferaseand reverse hydrolytic activity of Pectinex ultra enzyme preparation was investigated to produce oligosaccharides and disaccharides.

2. MATERIALS AND METHODS

2.1. Chemicals

Pectinex ultra SP-L commercial enzyme preparation from *Aspergillus aculeatus* purchased from Sigma-aldrich. Carbohydrates (glucose, mannose, lactose, lactulose, melibiose, sucrose, maltose, maltulose, palatinose, cellobiose, trehalose, turanose) were from Sigma-aldrich. All other chemicals are analytical grade and purchased either from Sigma-Aldrich or Reanal (Hungary).

2.2. Bioconversion

Oligosaccharide and disaccharide synthesis was investigated at 60 °C and pH 5.5 (Soerensen buffer) in a tube containing 1 ml of substrate solution with 30 g/100ml carbohydrate concentration. The tubes were kept in thermal bath. The reaction was starting by adding of 26 µl enzyme preparation. Samples were taken at time intervals and boiled for 10 minutes for stop the reaction before analysis.

2.3. HPLC method

The respective saccharide concentrations in the sample solution were determined using Thermo Scientific Corporation Surveyor HPLC system. The system is consisted of Surveyor pump with 4 channel, Surveyor automatic injector, Surveyor RI detector and Aminex HPX-87H column. Parameters of measurement: time of running was 25 minutes, eluent was 0,005 Nsulphuric acid, injected volume 10 ulfrom the (10 times) diluted samples, the temperature of the column and detector was 45 °C, the flow was 0.6 ml/min.

2.4. TLC method

For qualitative separation of sugars, TLC silica gel 60 pre-coated aluminum plates (20*20 cm) (from VWR) was used as stationary phase. Mobile phase was chloroform/acetic acid/water (30/35/5). The staining solution consist of 0.3 (w/v)%N-(1-Naphtyl)-ethylen-di-amin-di-hidrochloride, 5 (V/V)% H_2SO_4, 94.7 (V/V)% methanol. 2 µl of diluted sample (0.1 - 0.5 (w/v)%) was put on plates. The running time was 2 hours.

3. RESULTS AND DISCUSSION

3.1. Glycoslyl transferase activities of Pectinex ultra

Pectinex ultra SP-L enzyme preparation has already used for catalyze oligosaccharide transfer reactions. Previous studies mainly deal with fructosyl- and galactosyl transfer activity of the preparation(Ghazi *et al.*, 2007, Aslan & Tanriseven, 2007, Csanádi & Sisak, 2008).

According to HPLC resultsglucosyl transfer reactions were catalyzedon cellobiose, maltose, palatinose and trehalose by the preparation (Figure 1A). These substrates have different composition and different types of glycosidic bounds between the monosaccharide units. The wide range of possible substrates may caused by the composition of enzyme preparation. According to the specification given by provider, Pectinex ultra contains pectinase, cellulase, hemicellulase, ß-galactosidase and ß-fructofuranosidase, but the glucosyl transferase activity of the preparation has not been studied yet.

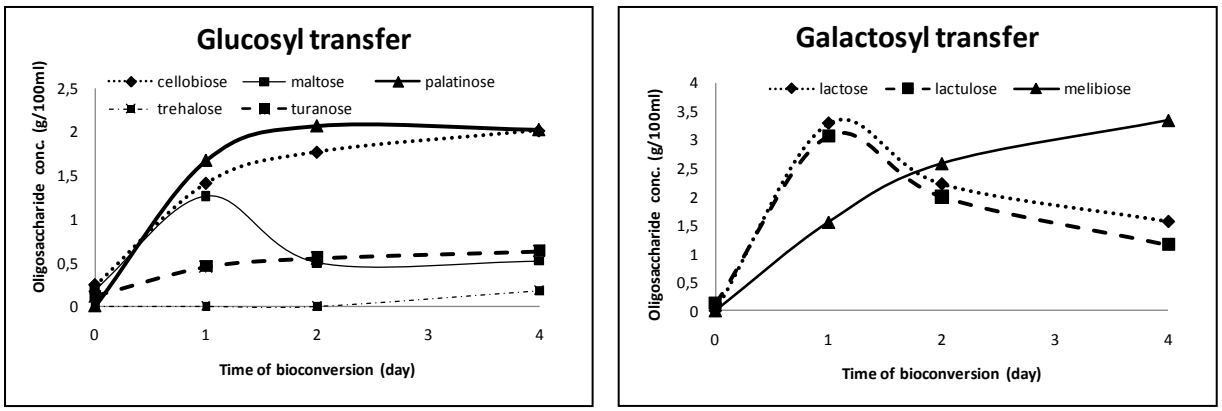

Figure 1: Oligosaccharide synthesis during glycosyl transfer (A: glucosyl transfer, B: galactosyl transfer) reaction on different substrates

Our results show, that the highest glucosyl transferase activity was on palatinose (isomaltulose, 6-*0*-α-D-glucopyranosyl-D-fructose) substrate. In this case 2.06 g/100ml oligosaccharide was synthetised on the second day of bionconversion. In case of cellobiose 2.02 g/100ml OS was produced on 4th day. On maltose substrate Pectinex shows an other

reaction course. The highest amount of OS-s (1.3 g/100ml) was detected on 1st day, but than it decreased very rapidly.

On trehalose substrate Pectinex shows hydrolytic activity and a very low transfer activity.

In our study galactosyl transfer reactions were monitored as well. This activity of the preparation was already reproted by some authors(Casteren *et al.*, 2000, Aslan & Tanriseven, 2007, Rodrigez-Fernandez *et al.*, 2011). Rodrigez-Fernandez and coworkers. (2011) have proved that ß-galactosidase from Pectinex is able to synthetise OS-s not only on lactose but also on lactulose. The Figure 1B shows that beside lactose and lactulose, melibiose is also a possible substrate of Pectinex. During the reaction the amount of glucose was double as amount of galactose so it can be concluded that on melibiose it was a galactosyl transfer reaction. In case of melibiose the course of reaction was different, than in case of lactose and lactulose. The highest amount of OS on lactose and lactulose (3.2 and 3.0 g/100ml) was measured on 1st day of bioconversion. On melibiose substrate it was detected (3.3 g/100ml) on 4th day.

Lactose and lactulose contain ß-glycosidic bound between the monosaccharide units, contrarily melibiose contain α-bound between the building glucose and galactose residue. This difference could be caused by the variance of bioconversion.

3.2. Reverse hydrolysis activity of Pectinex ultra

Reverse hydrolysis effect of Pectinex was analyzed on many different monosaccharides. 3. figure shows, that the preparation is able to synthetise mannose-disaccharides on mannose. The concentration of mannobiose was 1.8 g/100mlon 4th day.

Other authors have already used mannose as substrate for reverse hydroysis reaction (Ajisaka *et al.*, 1994, Singh *et al.*, 2000, Athanasopoulos *et al.*,2003). Ajisaka and coworkers (1994) synthetised with α–mannosidase from *A. niger* a many type of linkage beetween mannose units and so many different carbohydrates: α(1-2)mannobiose, α(1-3)mannobiose, α(1-6)mannobiose, α(1-2)-mannostriose and man-α(1-2)-man-α(1-6)-man oligosaccharide. Athanasopoulos and coworkers (2003) used a specific α-mannosidase from *Aspergillus phoenicus* for reverse hydrolysis which has a good regioselectivity and act just on α(1-6) bounds. This enzyme synthetise disaccharides and oligosaccharides with α(1-6) bound. Partially purified α-mannosidases from jack been, almond and limpet were also used for synthesis reaction by Singh and coworkers (2000)

Pectinex shows reverse hydrolysis reaction on glucose substrate as well (Figure 2). In case of glucose less amount of disaccharide was detected (1.4 g/100ml) but during the bioconversion a little amount of oligosaccharide (0.2 g/100ml) was produced as well. Previous studies have proved the reverse hydrolysis effect of many enzymes on glucose, for instance cellulase, α–amylase, glucoamylase, ß-glucanase, α-glucosidase (Pestlin *et al.*, 1997, Gama *et al.*, 1998, Chitradon *et al.*, 2000, Malá *et al.*, 2000, Meulenbeld *et al.*, 2002). *A. niger* glucoamylase (Pestlin *et al.*, 1997) carried out reverse hydrolysis reaction not only on glucose but also on arabinose, mannose fructose, xylose etc. *B. stearotermophylus* α-glucosidase (Malá *et al.*, 2000) was able to produce maltose, kojibiose, nigerose, isomaltose in glucose solution and heterooligosaccharides were obtained both with mannose and xylose.

In our study other monosaccharide substrates were also tested for reverse hydrolysis. Galactose, sorbose, rhamnose, fructose, arabinose, xylose are not substrates of reverse hydrolysis catalyzed by Pectinex. With TLC method no disaccharides or oligosaccharides were detected.

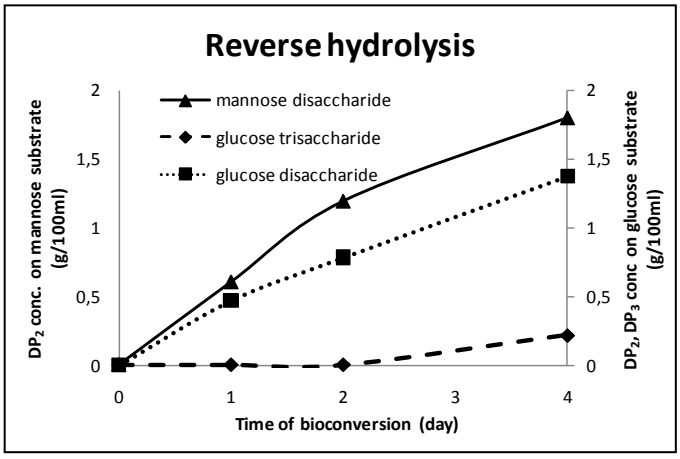

Figure 2: Disaccharide synthesis on glucose and mannose substrate and OS synthesis on glucose substrate during reverse hydrolysis reaction

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MONITORING OF CHEMICAL PARAMETERS OF QUALITATIVE PASTA SAMPLES CONTAINING DIFFERENT AMOUNTS OF MILLET FLOURS DURING STORAGE EXPERIMENTS

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SUMMARY
In the pasta technology the use of the (Panicum miliaceum L.*) millet's flour may be advantageous in several aspects. We can create safe and - in terms of nutritional value - qualitative pasta products without the use of eggs due to its pleasing yellow color. The millet contains no gluten, thus appropriate pasta structure not formed without the use of additives. In our work, flours used in course of traditional pasta production were supplemented with different quantity of millet flour. We were looking for answers how to change the pasta's chemical parameters (moisture content, pH value, acid value and soluble polyphenol content) during our storage experiments if we increase the millet flour amount.*

1. INTRODUCTION

The millet increasingly receives place in the organic farming nowadays thanks to the promotion of nutrition's reform. Millet's short growing season and versatile utility increase its value. Millet has a cholesterol lowering effect which is supported by the result of several animal experiments. Millet contained diet has many benefits in diabetes therapy (Rajasekaran et. 2004), as well as plays an important role in the protection of membranes. Millet contains very much of silica, also contains fluorine, iron, magnesium, calcium, zinc, potassium, manganese. In addition it is high in vitamins (especially in the B-group vitamins) (Cubadda et al., 2009; Shaidi and Chandrasekara, 2013). The millet is gluten-free, so people suffering in coeliac disease can be eaten also, but clean millet is difficult to produce in our country. Millet is a significant source of silicon, due to its high silica content. Phenolic compounds in millets are found in the soluble and insoluble-bound forms. Free phenolic acids are found in the outer layers of the kernel (pericarp, testa, and aleurone), whereas the bound phenolic acids are associated with the cell walls (Dykes and Rooney, 2006; Hahn et al., 1984; Heim et al., 2002).

The pasta-technology use in case of millet – which has very high nutritive values – is negligible in our country although several endemic could be prevented with its continuous consumption.

2. MATERIALS AND METHODS

2.1. Materials

All flours used for processing pasta were purchase from Hungarian market. Dry pastas were made of different flours and water. The codes of dry pastas and their flour compositions are shown in **Table 1**.

The pastas with different flour composition were made without egg. We could not produce pasta made of 100% *P. miliaceum* flour because it is a gluten free flour and we didn't want to add any food additives to dry pasta. Each pasta sample was manufactured with spindle shaped. These pasta products were made by small-scale technology and dried by the same drying method (40-60 ^{0}C). The drying parameters can be seen in **Table 2.** The duration

of the storage process was 12 months, and the samples were taken on the first day of each month. The storage conditions were: 20-25 ^{0}C, relative humidity 75-85%.

Table 1: Dry pasta sample codes

Flour composition of pastas	Dry pasta sample codes
100 % *T.durum*	Td0
100 % *T. aestivum*	Ta0
70% *T. durum* - 30% *P. miliaceum*	70Td 30Pm0
60% *T. durum* - 40% *P. miliaceum*	60Td 40Pm0
50% *T. durum* - 50% *P. miliaceum*	50Td 50Pm0
70% *T. aestivum* - 30% *P. miliaceum*	70Ta 30Pm0
60% *T. aestivum* - 40% *P. miliaceum*	60Ta 40Pm0
50% *T. aestivum* - 50% *P. miliaceum*	50Ta 50Pm0

Table 2: Parameters of the pasta drying

Drying period (hour)	Temperature (^{o}C)	Relative humidity (%)
0.00	36	70
2.00	50	72
4.00	59	68
6.00	64	62
8.00	65	55
10.00	54	45
12.00	47	34
14.00	44	20

2.2. Methods

The moisture content was determined in a drying oven. The oven was thermostatically controlled by a heating chamber capable of maintaining a temperature of 110 ^{0}C ± 5^{0}C.

The MSZ 6369-11:1987 standard was used to determine the pH and acid values of the samples.

Each flour, flour mixture and dry pasta products stored one year were sampled. The samples were homogenized and the extracts (0.10 g ml^{-1} in water after centrifugation for 10 min at 4 ^{0}C at 10,000 rpm) were made. Chemicals were purchased from SIGMA-ALDRICH Co. and REANAL Finechemical Co.

Soluble polyphenol content was measured by the colorimetric method with Folin & Ciocalteu's phenol reagent (Singleton & Rossi, 1965) and the results were expressed in Gallic Acid Equivalent (GAE) value (mmol gallic acid dry weight of pasta g^{-1}).

All analyses were performed at least in quintuplicate. Standard deviation was within ±5%.

3. RESULTS AND DISCUSSION

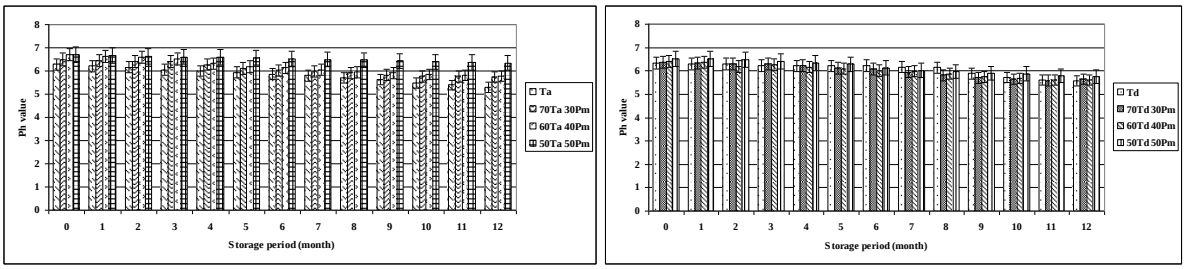

Figure 1: pH value of pastas made of T. aestivum, T. durum and P. miliaceum flour mixture

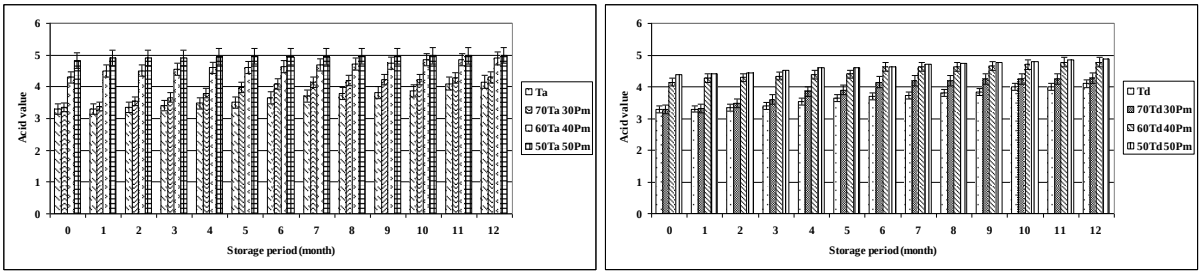

Figure 2: Acid value of pastas made of T. aestivum, T. durum and P. miliaceum flour mixture

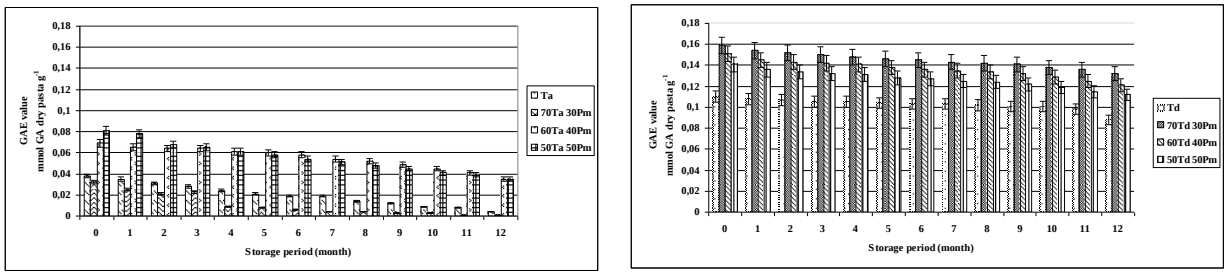

Figure 3: Soluble polyphenol content of pastas made of T. aestivum, T. durum and P. miliaceum flour mixture

During storage, we observed that the pH values (**Figure** 1) gradually decreased, while the acid values (**Figure** 2) gradually increased in the test samples. Both the pH and the acidity values were higher in samples in which millet flours was represented. The more the millet in the individual pasta samples, the higher the studied parameters. As a result, because of the increased acid value specially, it is necessary to reduce the shelf life of the samples which containing millet as against the pastas which are made from traditional flours only. Gradual reduction of the soluble polyphenol content (Figure 3) was observed during storage. However, we can conclude that the samples containing millet have higher soluble polyphenol content. This has a huge significance, because remarkable part of the valuable polyphenolic compounds's water-soluble fraction can be escaped to the hot water during the culinary processing of pasta.

ACKNOWLEDGEMENT: *The authors acknowledge the financial help of the GOP-1.1.1-09/01 and for Füri Pasta and Food Marketing Ltd.*

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COMPARISON OF MORPHOLOGICAL AND INNER CONTENT PARAMETERS OF DIFFERENT BEETROOT (*BETA VULGARIS* L.) VARIETIES

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SUMMARY
The beetroot typically is on the table in winter in from of pickles or juice but from hygienically due to its healthy parameters it would deserve more common consumption. Its curative effect in great part due to the numerous vitamins and mineral content and also contains C-, B vitamins, beta carotenes among others, in addition beetroot is rich in iron, copper, folic acid, magnesium, calcium. Its excellent antioxidant feature has been proved by animal experiments that the free radical produced in the liver can be neutralized by the antioxidant active substance. Besides of its numerous positive effects the surveys show that the beet plays a promimentl place order of the plans can accumulate soil pollutions. With using of our results we could compare the difference between the morphological and some inner content of two beetroot varieties.

1. INTRODUCTION

The beetroot is native to the Mediterranean area and ancestor of wild shape of *Beta vulgaris* L. var *maritime*. The Greek and Romans already knew, have cultivated in the II-III. centuries and used for food. It came to known in the XVII. century in Hungary.

The beetroot is one of the most then powerful vegetables regards antioxidant capacity. The polyphenol content for the total dry matter is 50-60 mol/g (Vinson et al., 1998; Kahkonen et al., 1999).

One study showed that the total phenol content of beetroot reduce in towards the inside in beet body. 50 % of the total phenol content is in the sell, 37% in the crown and 13% in the body. Betalaine content of the crown is 32% and betalaine content of the meat is 14% (Kujala et al., 2000). The valuable colour contents of the beetroot are nitrogen-containing, water-soluble pigments, these are the betalaines. These can be divided into two main groups; the red betacyans and the yellow betaxanthins. Next to the red colour material the beetroot contains anthocyanins which are responsible for the shades of red. The varieties of anthocyanidins are the hydroxy derivative of the flavilium cations therefore these are belong to the group of polyphenols. The pigments of the red and yellow beet have been proved that the red components have larger free radical scavenging capacity than the yellow's and it increases fluently with the fluid of the pH (Pedreno, 1999).

Among the components of the beetroot red component has the most important physiological effect. Research results have been published from the bacteriostatic effect of beet extracts by Sapiro (1963) and from the healing properties of beetroot in case of the inhibition of malignant cell growth by Ferenczi (2011). Clinical research results of Kapadia and his colleagues (2011) show the growth of prostate and breast cancer cells slow down for the betacyanin effect with 12%.

2. MATERIAL AND METHODS

2.1. Plant materials

In the experiment the autumn sowing of Cylindre and Alto F1 beet varieties are realized in conventional soil by the Experimental and Research Farm of Department of Vegetables and Mushroom Growing. Following the harvest of 10-10 plants per varieties the beets were washed, cleaned and we have been carried out the morphological investigations. Thereafter the beets were peeled and chopped. The rubble was made from comminuted raw material with mixer and the extraction was realized by mechanical process, compression.

2.2. Analytical methods

The determination of soluble solids content has happened with using of the refractometric measurement method (DBX ATAGO ATAGO and PR-301 models). Three independent measurements were realized in case of each samples.

The colour measurement was realized with Konica Minolta CR 400 type handheld digital colour measuring device which is on the base of the CIELab system. The lightness factor (L*), the a*coordinate indicates (the transition from red to green) and the b* (from blue to yellow) were determined.

The determination of the polyphenol content has been carried out with the method of Singleton and Rossi (1965). Applied measurement device was the Hitachi U-2900 spectrophotometer to the spectrophotometric measurement.

The determination of the antioxidant capacity happened with the FRAP method. The determination of FRAP value was realized with Benzie and Strain method (1996).

The determination betacyanin and betaxanthin content have been carried out according to the spectrophotometric method by Castellar and his colleagues (2003) and Stintzing and his colleagues (2005). The absorbency of the sample solutions to 2ref% diluted has been measured on 535 nm and 484 nm. Distilled water was used instead of blank samples.

3. RESULTS

In the comparison of morphological characteristics of two beetroot varieties the average weight of the 10 pieces Cylindra beets were 3.955 kg and the average weight of the 10 pieces Alto F1 beets were 2.985 kg. The average length of the 10 pieces Cylindra beets were 59 cm and the average length of the 10 pieces Alto F1 beets were 49.35 cm.

In case of the dry matter content of the investigated beet varieties the Cylindra beets showed higher values, the maximum value was in the end of the body. By the Alto F1 variety the largest dry matter content was in the middle of the body (Table 1).

Table 1: Dry matter content of the investigated beetroot varieties

Vatietis	Cylindra			Alto F1		
Body	Top	Middle	End	Top	Middle	End
Ref%	10,2	9,9	12,3	4,4	7,5	6,4

The Table 2 shows the results of the colour measurements. In case of the Cylindra the L* and the a* values were the highest in the top of the body and the values reduce towards the end of the body. The b* value was the highest in the middle of the body, slightly lower in the top of the body and the lowest b* value was in the end of the body. In case of the Alto F1 the L* values were the same in the middle and end of the body, and this value was slightly lower in the top of the body. The a* and the b* values were the highest in the middle of the body and in the top of the body the lowest.

By the Cylindra beets the polyphenol content was significantly higher, about double than by the Alto F1 beets (Figure 1). In case of the Cylindra beets the polyphenol content almost the same in the top and middle of the body and in the end of the body this value was slightly higher. In case of the Alto F1 the polyphenol content increased from the top to the end of the body.

Table 2: L*, a* and b* values of the investigated beetroot varieties

Varieties	L*			a*			b*		
	Top	Middle	End	Top	Middle	End	Top	Middle	End
Cylindra	20,74	20,52	19,57	2,47	2,35	2,13	1,56	1,65	0,64
Alto F1	20,20	20,47	20,47	1,03	2,52	2,22	1,31	1,63	1,48

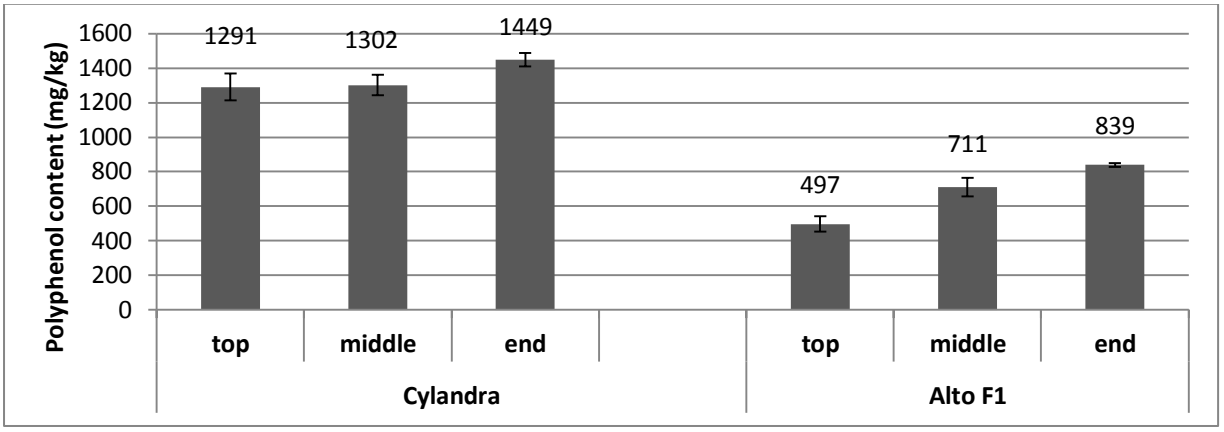

Figure 1: Polyphenol content of the investigated beetroot varieties

By the Cylindra beets the antioxidant capacity was significantly higher than by Alto F1. In case of both beetroot varieties the lowest anthocyanin content values were in the middle of the body and in case of the Cylindra beets the highest value was in the end of the body, in case of Alto F1 beets in the top of the body (Figure 2).

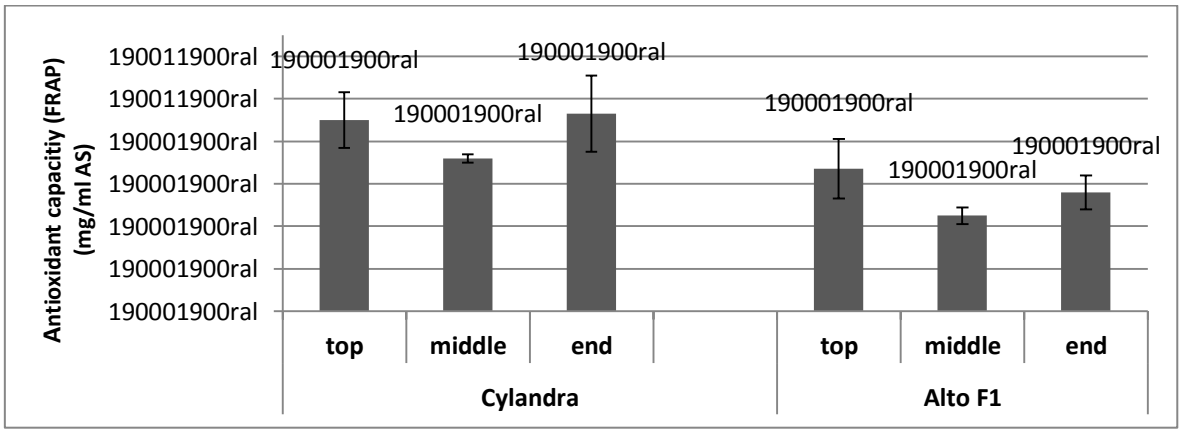

Figure 2: Antioxidant capacity of the investigated beetroot varieties

In case of the determination of the betanin content of the investigated beetroots our results showed both betacyanin and betaxanthin contents were higher by the Cylindra beets what the 3 and 4 Figure shows.

4. CONCLUSION

The results of the morphological investigations showed the Cylindra variety produced more favourable crop parameters than the Alto F1 variety.

In case of the investigated inner parameters more favourable values could be shown for the Cylindra variety. The distribution of the inner parameters were not equable in the root body. Based on these measurements the cultivation of the Cylindra variety is recommended, but this was a preliminary experiment so further measurements are needed such as element content and colourless flavonoids to be relevant this statement.

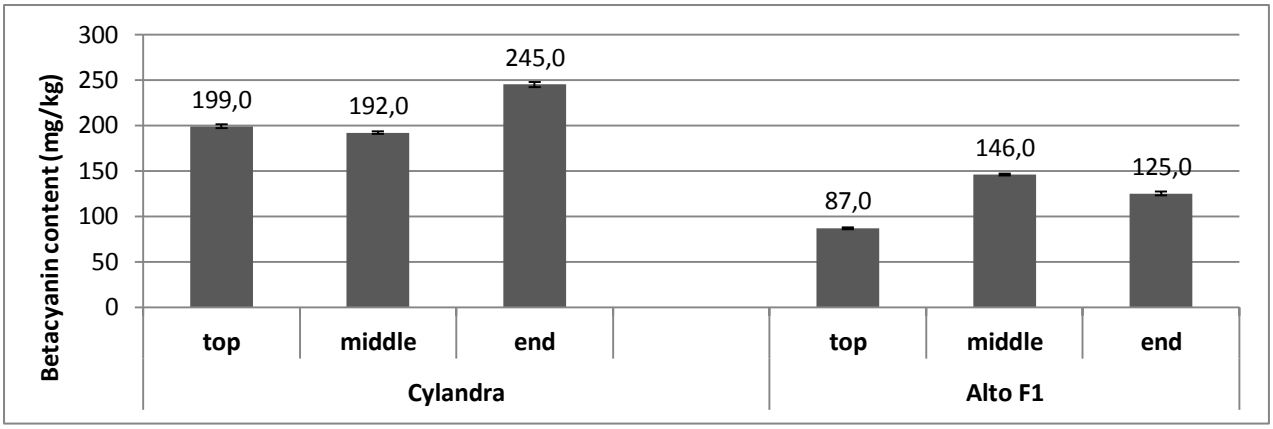

Figure 3: A betacyanin content of the investigated beetroot varieties

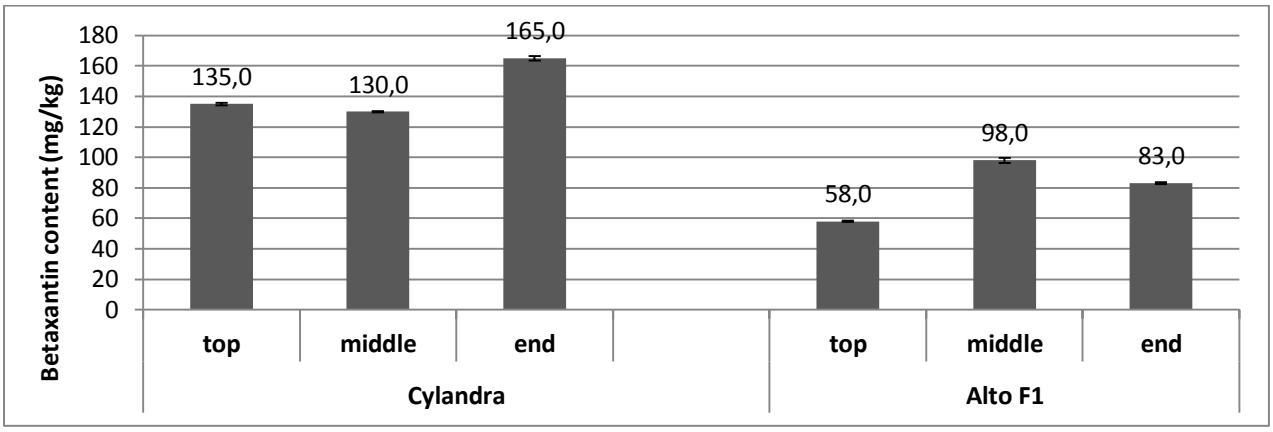

Figure 4: A betaxanthin content of the investigated beetroot varieties

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SECTION 3: Food technology; Food economy, logistics and consumers

EFFECT OF HIGH HYDROSTATIC PRESSURE ON CHARACTERISTICS OF DRY SAUSAGE DURING RIPENING

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SUMMARY
Effects of high hydrostatic pressure were studied on the physical, chemical, microbiological and sensory properties of dry ripened sausage. Raw sausages were manufactured and pressurized before smoking, after smoking and after ripening. HHP treatment efficiently reduced the aerobic cell count of the products. Results showed that HHP treatment before ripening enabled to shorten the drying period of sausages. HHP caused changes in colour, however the difference between Control and HHP sausages nearly disappeared by the end of ripening. Pressurization did not affect lipid oxidation of the products. Intensity of sausages' aromatic flavour decreased and became slightly acidic due to pressurizing before ripening. HHP treatment changed the shape of sausages which change diminished noticeably during storage. Results showed that the sequence of smoking and pressurizing was irrelevant, however, sequence of ripening and HHP affected the characteristics of sausages.

1. INTRODUCTION

High Hydrostatic Pressure (HHP) treatment is one of the successful recent methods for preserving food. High pressure efficiently inactivates pathogens and microorganisms responsible for spoilage without significantly changing the technofunctional properties of the product (Balasubramaniam et al. 2008; Balny, Masson 1993). HHP ensures microbiological safety, however, the treatment can affect the product's physical and chemical properties in different ways, depending on the type of food. This makes necessary to know the effect of high hydrostatic pressure on the characteristics of different kinds of food, such as meat and meat products.

It is important to know HHP's effect on proteins, being one of the main constituents of meat and meat products. Protein denaturation caused by HHP may affect the products' colour, texture or flavour (Sikes et al. 2009; Ruiz-Capillas et al. 2007). HHP can also affect the lipid oxidation of meat products (Cheah, Leaward 1996).

The aim of the study was to investigate how the sequence of HHP treatment, smoking and ripening affects the quality and characteristics of dry ripened sausages.

2. MATERIALS AND METHODS

When investigating how the sequence of technological steps affect the properties of sausages, microbiological aspects and effects on colour, lipid oxidation, pH, water activity and sensory properties were studied.

Sausages contained pork meat, lard and several spices, such as salt, paprika, pepper and garlic. After chopping, mixing and mincing raw materials the paste was filled into natural casing. Samples were divided into three groups: HHP before smoking, HHP after smoking and HHP after ripening. Sausages were pressurized at 600 MPa for 5 minutes. During HHP treatment sausages were kept in vacuum packaging which was removed after pressurization. Smoking lasted 24 hours long and the temperature of smoke was 20 °C. Ripening lasted 4 weeks long at 11 °C temperature and samples were examined every 4 days.

All samples were pressurized in a RESATO FPU-100-2010 high hydrostatic pressure equipment. Colour measurements were performed by a Minolta CR-200 tristimulus colorimeter, the rate of rancidity was determined by TBA test. Water activity and pH were measured using Novasina Labmaster-aw and Testo 206-pH2 devices. In the microbiological examinations total aerobic cell count was determined.

3. RESULTS AND DISCUSSION

As an effect of HHP treatment the aerobic cell count of sausages decreased nearly by two orders of magnitude. Therefore microbiological safety could be increased by pressurization (Figure 1.).

Control sample's pH was significantly lower than the pH of pressurized sausages. The reason is that HHP efficiently inactivated lactic acid bacteria responsible for pH reduction as a result of acid production (Figure 2.).

The rate of Control sample's pH reduction is typical of sausages fermented by starter cultures, even though no bacteria were added to the product. This was caused presumably by the original microflora of raw materials.

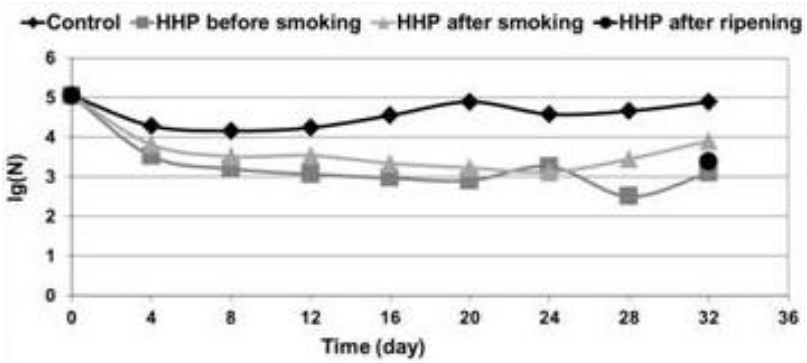

Figure 1: Change of sausages' microbiological status due to HHP treatment during storage

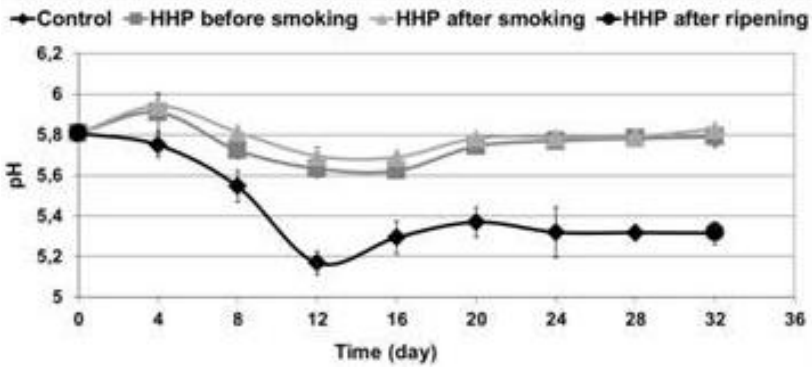

Figure 2: Change of sausages' pH due to HHP treatment during storage

Water activity of Control and HHP sausages decreased similarly (Figure 3.). Therefore drying period of sausages can be shortened by pressurization considering the Control sample's significant pH reduction which decreases water-binding ability of meat.

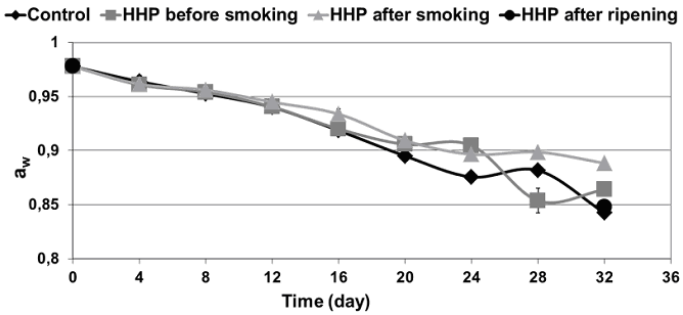

Figure 3: Change of sausages' water activity due to HHP treatment during storage.

High hydrostatic pressure did not change significantly colour on the external surface of sausages (Figure4 and Figure5.).

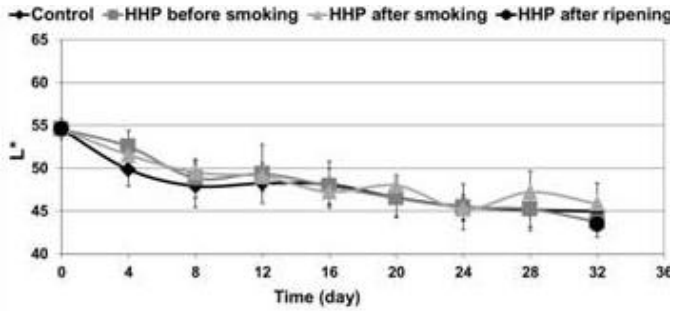

Figure 4: Change of L* values of the external surface of sausages due to HHP treatment during storage.

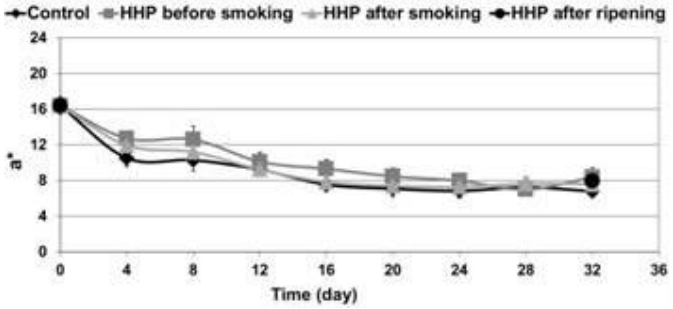

Figure 5: Change of a* values of the external surface of sausages due to HHP treatment during storage.

After cutting the samples, results showed that due to HHP treatment L* values increased (Figure 6.) and a* values decreased on the internal surface (Figure 7.). The b* was not affected by pressurizing. Contrast between lard and meat particles decreased also, however at the end of ripening there was no noticeable difference between Control and HHP sausages.

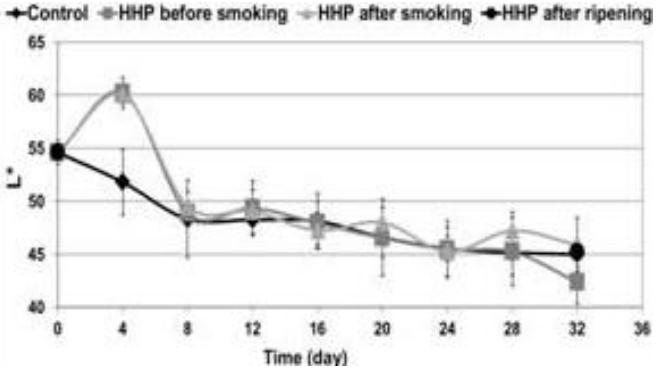

Figure 6: Change of L* values of the internal surface of sausages due to HHP treatment during storage.

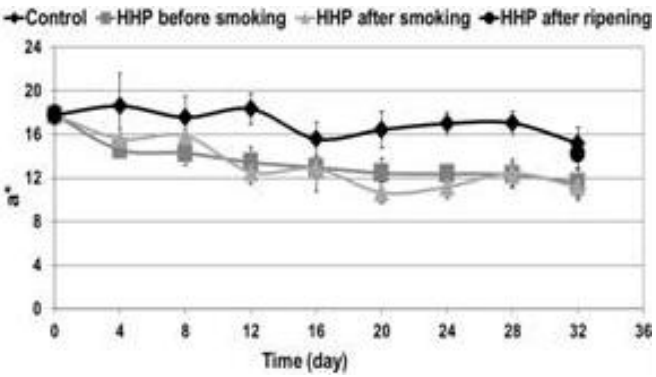

Figure 7: Change of a* values of the internal surface of sausages due to HHP treatment during storage.

Pressurization did not cause significant changes in the lipid oxidation of the samples.

Taste of sausages pressurized before or after smoking became less aromatic and acidic than the Control sample. It was caused by HHP's inactivating effect on bacteria producing aromatic components and lactic acid. There was no difference between the taste of Control sample and sausages pressurized after ripening.

HHP treatment before ripening caused a noticeable change in the shape of sausages, however the rate of this change decreased during the storage.

4. CONCLUSIONS

The sequence of smoking and pressurizing did not have a significant effect on the characteristics of sausages, however, sequence of HHP and ripening affected the quality and properties of the product.

HHP treatment at the beginning of ripening enabled to shorten the time of drying and increased microbiological safety, however, the products taste became less savoury.

Pressurizing at the end of ripening also ensured the microbiological safety, there were no changes in taste and shape, but in this case the drying period cannot be shortened.

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PARADOXES IN THE FIELD OF FOOD POLICY

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SUMMARY

This lecture would like to present some of the paradox related issues concerning the Food Economy. Issues such as: - the contradiction between the wastage of food and hunger,
- the over nourishment, and its effects on the society due the related influence on general health,etc.

INTRODUCTION

According to Trichopoulou[1], the concept of "food policy" can be defined as the system of conditions and tools of "nutrition policy"[2] accepted with international consensus and working with governmental guarantee. In this sense, food policy means the entirety of various government policies. However, in my lecture I am not going to focus on government actions; instead, I am going to give examples of the contradictions between the behaviour of the consumers affected by these actions and the interest of the society as a whole.
I would like to make two further remarks. Since Professor Trichopoulou created the concept and system of food policy, a shift of emphasis can be observed in the judgement of the role of nutrition.

1./ Food is no longer looked upon only as a product which satisfies our needs of nutrition and enjoyment but increasingly as a product maintaining health. This can fundamentally determine the practice of food product development.[3]

2./ Providing food as a complex activity has thus become the subject of novel integration relationships and is more and more closely connected to "health industry".[4]

PARADOX 1. PARADOX OF WORLDWIDE STARVATION AND FOOD WASTE

This is probably agreed to be the greatest socio-political and ethical paradox against mankind. Only estimates are available for the extent of starvation as well as for the amount of food waste. According to these, one in eight people is underfed in the world[5] and every year approximately 18 million people die of hunger.[6] Starvation cannot be linked exclusively to a continent or a country because there are strata suffering from malnutrition or insufficient nutrition in each country (naturally, their proportion is different compared to the entire population). It is easier to localize food waste; it is more typical in developed countries than in medium-developed ones, and it is probably insignificant in underdeveloped countries.

Several estimations are known for quantifying food waste. Every year the amount of food still edible but ending up as garbage comes to 1.3 billion tons worldwide and 400 thousand tons in Hungary[7], while according to other sources around 2 billion tons per year in the USA and in Europe. This means mostly food which is thrown out. As stated in a paper on this topic,[8] in the developing world loss arises between the land and the stores, while in the developed world – where waste is greater[9] – in the stores and in the homes. As regards the phase of raw material production, the data on the loss of vegetable and fruit production are particularly instructive, here – as the already cited source claims – products which do not comply with the quality standards of the store but are otherwise fit to be eaten may be wasted.[10] This figure implies a loss of 45 million tons of fruits-vegetables in Europe, as for the number of apples, 3,700 billion.[11] A leading industrial expert is of the opinion that the problem of starvation could be alleviated by developing 1./ production and infrastructure; 2./ processing and packaging; 3./ retail trade.[12]

In my opinion waste is generated mainly in the households. This can take several forms, its most common form is over-consumption. Later on I am going to comment on ensuing obesity as an epidemic disease separately. Some publications also regard the by-products and waste which arise necessarily during food technological processes as "food waste". This is a professional mistake because normally and in Europe these products are hardly suitable to be eaten by humans. It is another matter – and also our best interest – that technological waste and loss should be reduced to a reasonably low level. However, relevant "recommendations" are not always original ideas. For example, Hungarian milk industrials scarcely need to be taught to use whey and buttermilk, by-products of milk processing, for nutritional purposes.[13]

At the same time food waste is also a serious environmental problem. In the cited article Tim Fox estimates that about 550 billion cubic meters of water was used for the production of food which ended up as waste. The packaging materials of wasted food thrown out as garbage impose a further burden on the environment (concerning which no calculations have been made).

This global paradox needs to be resolved urgently because the population of the Earth is increasing drastically and is expected to reach 9.5 billion by 2020, which means that compared to the current situation, 3 billion more will have to be provided for and fed by the Earth. The EU Waste Framework Directive[14], which also aims to decrease food waste, put together a program package for the solution of this paradox with tasks to be performed in a hierarchical system. FAO launched the Save Food initiative on food loss and waste reduction, while food-saving campaigns are organized under the name of Food Bank in 22 European countries. In addition, the Élelmiszer Online web journal describes numerous valuable civilian and commercial initiatives, which all aim to achieve the conscious and planned reduction of food waste.[15] The official Hungarian initiative put forward in the EU Council of Ministers of Agriculture, calling for the global assessment of the situation and for taking the necessary measures, particularly needs to be pointed out.[16]

In Europe 79 million people live below the poverty line and 30 million do not eat appropriately.[17] In Hungary approximately 1 million people are in need of food aid which has come from the EU since 2006 and which was expected to show a significant decrease by 2012.[18]

PARADOX 2. OBESITY: THE PERSON'S FREEDOM TO BE OVERWEIGHT AND THE SOCIETY'S OBLIGATION TO AVERT THE CONSEQUENCES

There is a further problem linked to the sphere of problems presented above, and this also presents itself as a serious paradox. This is the relationship between obesity as an epidemic disease and the various ensuing nutrition-related diseases (hypertension, circulatory diseases, diabetes, etc.).

Halmy (2008) claims[19] that the world epidemic of obesity currently affects 310 million people in the world. The alarmingly high incidence (1.2 billion) of pre-obesity, that is being overweight, also contributes to the occurrence of obesity. The health significance of obesity is due to its associated diseases.

Obesity and overweight can be considered to be an epidemic in Hungary, too, in the light of the fact that 60 per cent of the adult population is obese or overweight, and this proportion continues to increase. There is a social reason for it: the consumption of food with more affordable prices and the lack of exercise. On a national average every adult consumes about 500 calories in excess per day. "Every hour 7 people die in obesity-related diseases and the society's extra expenditure caused by the disease amounts to more than HUF 30 billion a year," said László Halmy, president of the Hungarian Society of Obesity Research.[21]

A significant proportion of overweight people belongs to the poorest and the least educated strata of the society.[22] Here the paradox appears as the contradiction between the civil (individual) freedom right to obesity and the all-around social responsibility to avert its consequences. To put it sharply, the contradiction can be worded as follows: "The individual has the freedom to eat in a healthy or unhealthy way. The individual's freedom lies in living a healthy or sick life depending on this choice, but if he/she "chooses" obesity and disease as its consequence, it is the obligation of the society to treat the disease and to avert the harmful consequences." This is unfair and unjust behaviour towards those who are ill through no fault of their own and from whose medical treatment huge sums are taken away. Just one figure: in the USA the costs of the medical treatment of obese people amounted to 190 billion dollars in 2005. Besides, obese people spent an estimated sum of 40-100 billion dollars on dietary products.[23] In the USA the health care costs for patients with diabetes resulting from obesity were 2.3 times higher than the health care costs of the non-diabetic population. In Hungary the health care expenditure for one diabetic patient was HUF 274 thousand in 2008, which corresponded to 0.5 per cent of the GDP.[24] Not to mention the social costs of other "epidemic diseases" caused by obesity, such as cardiovascular diseases, hypertension etc.

Under current regulations, these preparations are subject only to being reported to the National Institute for Food and Nutrition Science. The Institute can give 4 answers in response to such a report, but in general it cannot investigate its veracity. Not long ago the result of a study conducted by the University of Szeged received much publicity as the composition of a weight control dietary supplement was found not to correspond to what had been reported.[25]

PARADOX 3. MORE SCIENCE ON THE ONE HAND, MORE MISCONCEPTIONS ON THE OTHER

More and more science-based information and results of discoveries are available concerning healthy nutrition. Yet the need for their application is not increasing, on the contrary, it seems to be decreasing. This is mainly due to the spreading of the so-called "alternative" nutritional trends, which place eating into a spiritual environment and order many prohibitions. These prohibitions are usually disguised in pseudo-scientific explanations, frequently with life-threatening rules.

Diet was regulated by religious rules in the past as well. This spiritual approach to diet might as well be appropriate, but nutritional sects take advantage of the half-educated consumers' ignorance. Consumers who are scientifically educated can distinguish relatively easily between diets formed according to the religious regulations of world religions and the "advice" of nutritional sects which vulgarize religious regulations. While the former ones are usually more lenient, the rules of nutritional sects contain much more prohibitions.

With respect to the topic of our conference it is important to know that the special norms of "nutritional sects" reject the consumption of industrially processed foods, while food industry has participated in producing foods according to the regulations of world religions for decades. A somewhat informed consumer – irrespective of religious beliefs – knows and appreciates the role of industry in offering the choice of "healthy", dietary ("free from") and antiallergic products, though consumer opinions about E numbers reveal how limited this knowledge is.

The fundamental cause of the contradiction lies in the receiving medium itself, which is bombarded with a wide variety of information through countless communication possibilities, and the consumer selects among them according to his/her readiness, level of intelligence, or even belief and state of being influenced. By using the interactive possibilities provided by Internet communication, anyone can become a nutritional and lifestyle consultant regardless of whether he/she possesses the necessary scientific grounding, or in the absence of this he/she does this as the "priest/priestess" of some nutritional "sect", or possibly as a simple follower or just in the capacity of a housewife.

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EVALUATION OF PRETREATMENT AND PARAMETERS OF PRE-DRYING ON MICROWAVE VACUUM DRYING COMBINED WITH HOT-AIR PRE-DRYING TECHNOLOGY

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SUMMARY

Microwave vacuum drying (MVD) combined with hot-air pre-drying is one of the newest method of mild fruit and vegetable processing. Combined MVD is a rapid and efficient drying method, which results unique characteristics, better consistency and improved nutritional quality of the product, than the hot-air dried ones.
Our aim was to develop the technology and the product, which includes the pretreatment steps, the optimization of the pre-drying and MVD parameters and the complex quality analysis of the product. In this study, the pretreatment steps, (soaking, blanching) and the parameters of pre-drying (temperature, dry mass content) was studied, aimed at the improvement of the color, consistency and nutritional quality of the product.

APPLICATION OF THE SMARTFRESH QUALITY SYSTEM ON PEARS

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SUMMARY
The active component of the SmartFresh Quality System is the 1-methylcyclopropene. By the application of the 1-MCP compound quality of the harvested pears can be longer preserved during the normal cold storage. In our work the effectiveness of the SmartFresh Quality System was investigated on 'Bosc Kobak' pears harvested at different times. The rheological changes and storage losses were measured. The effectiveness of 1-MCP compounds depends on many variables, but our results show that the optimal harvest date and the condition of the harvested fruits are the most influential factors.

1. INTRODUCTION

1-MCP is an ethylene action inhibitor that has been used after harvest to retard ripening in a range of fruits (Sisler and Blankenship, 1996, Sisler and Serek, 2003 and Watkins, 2006). In pear fruit, 1-MCP treatment has been reported to decrease softening, color development, respiration rates, and ethylene production (Baritelle et al., 2001, Argenta et al., 2003, Kubo et al., 2003, Hiwasa et al., 2003, Calvo and Sozzi, 2004, Calvo and Sozzi, 2009, Calvo, 2004, Ekman et al., 2004, Trinchero et al., 2004, Hitka et al., 2006 and Mwaniki et al., 2005).
'Bosc Cobak' is one of the three most important winter pear cultivars grown in Hungary. 'Bosc Kobak' pears are normally harvested from middle September to middle October and cool-stored until marketing, generally within 90–120 days to avoid losses due to the development of superficial and senescent scald and internal breakdown. Ethylene produced by the fruit during storage can exacerbate the incidence of these physiological disorders, and 1-MCP is highly effective to control or reduce their incidence (Du and Bramlage, 1994, Watkins et al., 1995, Whitaker and Solomos, 1997, Ju and Curry, 2000, Bower et al., 2003, Ekman et al., 2004 and Gapper et al., 2006).

Although postharvest application of 1-MCP provides valuable benefits, it is challenging to obtain normal softening and ripening in 1-MCP-treated 'Bosc Kobak' pears if the treatment is performed immediately after harvest. The 1-MCP treatment appears to reinforce the natural characteristics of European pears which are resistant to ripening after harvest, and require a period of cold storage or ethylene exposure to induce ripening (Villalobos-Acuña and Mitcham, 2008).

2. MATERIAL AND METHODS

'Bosc Kobak' pears (*Pyrus communis* L.) were harvested from a commercial orchard in Bodrogköz area, Hungary. There were 2 different harvest dates (mm.dd.yyyy.): 09.11.2012. and 09.16.2012.

The 1-MCP treatments were performed 2 days after harvest at Kiskunfélegyháza in a cold storage. Core-temperatures of the pears were below 5°C during the treatment, while the room temperature was set to a constant value. Fruits were exposed to 1-MCP (SmartFresh®, AgroFresh, Philadelphia, USA) for 24 h, the concentration of 1-MCP was 625-650 ppb. 1-MCP treatments were carried out in airtight storage rooms with continuous ventilation, as described in the requirements of the SmartFresh Quality System.

Thirty fruits were used to analyze different maturity indices (firmness, soluble solids concentration and starch degradation, black seed percentage) at the beginning of the experiment. Some of the samples received a red mark, they did get 1-MCP treatment (Control), while the green marked group means the treated samples (SF). After the treatments fruits were immediately put to storage and kept at 20 °C for 10 days or stored at −0.5 °C and 95% RH for 60, 120, or 180 days, respectively. During the cold storage carbon dioxide level was no higher than 1.5 vol%. After cold storage, fruits were transferred to a room at 20 °C for 1 or 7 d for further ripening and fruit firmness, soluble solids concentration and ethylen production measurements were assessed. Measurements were carried out in the Department of Postharvest Science and Technology.

3. RESULTS

Table 1. shows the postharvest quality of 'Bosc Kobak' pears at two different harvest times. Percentage of black seeds indicates the action of ethylene in the fruits. Higher ethylene production leads to maturation which can also be determined by the measurement of starch content. While firmness was similar at the two harvest times, sugar content, starch content, percentage of black seeds and ethylene productions show us that pears harvested later were in higher maturity stage. Using the SmartFresh Quality System after 10 days of shelf-life the fruits kept their firmness and their ethylene production was blocked.

Table 1: Quality of the pears at harvest time and after 10 days of shelf-life

		Avg. Firm. kg/cm²	*Brix %*	*Black seed %*	*Starch (1-6)*	*Disorders %*	*Ethylene prod (uL/kgh)*
Harvest date: 09.11.2012. **Application date: 09.13.2012.** **Shelf-life date: 09.24.2012.**	***Initial***	***14,22***	***14,39***	***28%***	***1,3***	***0%***	***-***
	Shelf Life Control	***2,92***	***15,42***	***100%***	***-***	***0%***	***2,47***
	Shelf life SF	***13,26***	***14,46***	***100%***	***-***	***0%***	***0,09***
Harvest date: 09.15.2012. **Application date: 09.18.2012.** **Shelf-life date: 09.29.2012.**	***Initial***	***14,52***	***13,60***	***58%***	***2,45***	***-***	***-***
	Shelf Life Control	***6,88***	***13,87***	***100%***	***-***	***0%***	***4,98***
	Shelf life SF	***11,22***	***14,39***	***100%***	***-***	***0%***	***0,18***

Table 2. represents the quality of control and 1-MCP (SF) treated 'Bosc Kobak' pears after 2, 4 and 6 months of storage and 7 d shelf-life. During the storage period firmness decreased only by 2-3 kg/cm^2. The real differences came from the shelf-life time between the treated and control groups. Fruits from the first harvest date were in better condition at the end of storage. After 4 months of storage ethylene production started again in the SmartFresh fruits which means that storage time could be extended by minimum 2 and maximum 4 months at this variety using 1-MCP. Another benefit of using SmartFresh Quality system is the decrease of storage disorders, it means by 20% more 1st class quality pears after a long storage time. Only 3 days difference in the harvest time can lead to 3 kg/cm^2 difference in firmness during the shelf-life. So if it's necessary to keep the firmness during the shelf-life (sell the product to fruit trader) its better to choose an earlier harvest time. But on the other hand if you would like to put the pears to the fresh market it is better if the firmness is closer to ready to eat quality so in this situation it is advisable to choose the second harvest time for storage in spite of knowing that the percentage of disorders can be higher.

Table 2: Quality of the pears after 2, 4 and 6 months of storage and 7 d shelf-life

	Harvest date: 09.11.2012.				*Harvest date: 09.15.2012*			
2, 4 and 6 months storage (ST) + 7 days Shelf Life (SL)	***Avg. Firm. kg/cm²***	***Brix %***	***Disorders %***	***Ethylene prod. (uL/kgh)***	***Avg. Firm. kg/cm²***	***Brix%***	***Disorders %***	***Ethylene prod (uL/kgh)***
2M Storage Control	***11,92***	***14,85***	***0%***	***3,01***	***10,82***	***14,83***	***0%***	***2,59***
2M Storage SF	***13,34***	***15,44***	***0%***	***0,24***	***12,80***	***14,49***	***0%***	***0,25***
2M ST + SL Control	***2,6***	***14,00***	***0%***	***11,47***	***2,02***	***15,42***	***0%***	***5,44***
2M ST + SL SF	***11,22***	***15,46***	***0%***	***0,81***	***7,76***	***15,40***	***0%***	***0,63***
4M Storage Control	***9,66***	***14,19***	***0%***	***4,74***	***11,68***	***14,53***	***0%***	***4,06***
4M Storage SF	***12,38***	***14,99***	***0%***	***0,85***	***12,90***	***15,18***	***0%***	***0,75***
4M ST + SL Control	***3,82***	***15,51***	***10%***	***7,62***	***3,22***	***14,68***	***40%***	***6,94***
4M ST + SL SF	***9,4***	***15,24***	***0%***	***1,31***	***6,44***	***15,05***	***25%***	***2,38***
6M Storage Control	***11,74***	***14,44***	***30%***	***4,41***	***11,02***	***14,64***	***32,5%***	***4,43***
6M Storage SF	***11,82***	***15,52***	***0%***	***1,08***	***12,2***	***16,57***	***7,5%***	***2,46***
6M ST + SL Control	***4,36***	***14,25***	***30%***	***8,44***	***3,88***	***14,19***	***30%***	***6,12***
6M ST + SL SF	***7,74***	***16,43***	***10%***	***6,57***	***4,32***	***16,21***	***10%***	***4,42***

4. CONCLUSIONS AND ACKNOWLEDGEMENTS

The optimal harvest time selection leads to better firmness, less storage disorders and better shelf-life quality. The starch content and the percentage of black seeds of the fruits are key issues of the long term storage. We suggest that the optimal firmness should not be lower than 14 kg/cm^2, the starch content not higher than 2 (1-6 scale) and the percentage of black seeds should not be higher than 30% for long-term storage. Using SmartFresh Quality system the ethylene production can be blocked for 2-4 months which can result better fruit quality after storage and longer shelf-life also.

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IMPEDANCE MEASUREMENT OF SOUS-VIDE AND HIGH HYDROSTATIC PRESSURE TREATED LONGISSIMUS DORSI OF PORK

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SUMMARY

The pork's Longissimus dorsi (prepared to be free of fat, ligament and connective tissue) was cooked sous-vide for 1,5 to 9 hours, treated with high hydrostatic pressure (HHP) and treated as well with the combination of the two methods above. The impedance and the phase of the samples were measured with a two electrode puncture-type sensor - using two precision LCR meters HP4284A and HP4285A in the frequency range of 20 Hz – 1 MHz and 75 kHz – 30 MHz respectively. After the analysis of the measured data we can state that impedance measurement can be a proper method to detect the effects and the changes in the samples due to the sous-vide and the HHP treatments. The longer the heat treatment and the higher the HHP treatment, the bigger the impedance value was with a change in the phase shift as well.

1. INTRODUCTION

Both high hydrostatic pressure treatment and the sous-vide technology as minimal processing methods are very effective in their field but beside the advantages we still face several problems. Among others the survival of psychrofil and psychrotolerant microorganismes or the incomplete inactivation of enzymes. The combined application of these technologies could be beneficial both on the quality and the shelf-life of the products. (Vaudagna, 2002)

In recent years high pressure technology has received considerable attention as a method of food processing and preservation (Ma-Ledward, 2004) However, pressure and low temperature heat treatment can also bring about changes in the structure of meat (Sun-Holley, 2010; Marcos et Al. 2010). To detect these changes an apropriate method is necessary.

The use of electrical measurements to study meat goes back to decades. (Damez et Al.,2007) The electrical impedance of biological – both plant, and animal – tissue gives a characteristic spectrum in the frequency range from 10 Hz up to 100 MHz. (Grimnes-Martinsen,2000). The impedance of biological tissues and in particular of meat depends on its structure, and on the ionic conductivity. This later is related strongly to the state of the meat. (Damez et Al.,2007). Impedance measurement can be a simple and non-destructive method to reveal differences between the diverse meat samples.

The aim of this study was to determine the impedance spectrum of meat treated with sous-vide and high pressure technology and to find the correlation of impedance parameters with time of sous-vide treatment and with pressure value of HHP.

2. MATERIALS AND METHODS

Two whole pork chops (Longissimus dorsi) were purchased in a wholesale supermarket (m_1= 3,14 kg; m_2= 2,85 kg). After being prepared to be free of fat, ligament and connective tissue each meat was cut into ten pieces transversally to the fibre direction. The pieces were cooked at 60°C sous-vide and treated at different high hydrostatic pressure for five minutes. (table 1.)

Table 1: The cut of the meat and the applied combination of treatments

4,5 h SV	**9 h SV**	**untreated ref.**	**9 h SV**	**4,5 h SV**
300 MPa HHP + 4,5 h SV	**600 MPa HHP + 9 h SV**	**600 MPa HHP + 4,5 h SV + 600 MPa HHP**	**300 MPa HHP + 9 h SV**	**600 MPa HHP + 4,5 h SV**

untreated ref.	**1,5 h SV**	**3 h SV**	**4,5 h SV**	**4,5 h SV + 400 MPa HHP**
600 MPa HHP	**1,5 h SV + 600 MPa HHP**	**3h SV + 600 MPa HHP**	**4,5 h SV + 600 MPa HHP**	**4,5 h Sv + 200 MPa HHP**

The magnitude and phase angle of impedance in the frequency range from 20 Hz to 30 MHz were measured on three different sides of the sample – on the top parallel and perpendicular to the fibres and on the side surface with two precision LCR meters – Hewlet-Packard 4824A and 4825A. Each spectrum was recorded three times. The measured spectra were open-short corrected in order to eliminate the stray capacitance and inductance above 100 kHz. The level of measuring voltage was 1 volt. The measurement was carried out with a two electrode puncture-type sensor. The electrodes penetrated 20 mm below surface. They were insulated along the length except 1 cm section on the end. The distance between the two electrodes was 1 cm, the electrical field strength is 100 V/m which is enough low not to cause physiological changes in the cell membrane. (Kaltenecker et Al., 2013)

The texture of processed meats were measured with a Stable Micro System TA-XT2 texture analyser. The maximal force on cylindrical head of 5 mm diameter during a rupture test and the maximal force on spherical head of 10 mm diameter under 10 mm deformation with 2 mm/s velocity was detetected.

Water loss was calculated from the initial and the after-treatment mass of meat samples measured with a KERN PLJ 750-3N precision balance.

3. RESULTS AND DISCUSSION

Typical impedance magnitude (Figure 1.A) and phase angle (Figure 1.B) spectra of treated meat in three various directions are in good agreement of literature data. The orientation of sample under measurement had slight effect on the magnitude of impedance and practically had no effect on phase angel. The measured impedance magnitude varied between 250 and 560 Ohm at 100 kHz in meat under various treatments. In general we can say that as the treatments were longer and stronger the impedance magnitude was higher.

The phase angle of the impedance magnitude in the function of frequencies showed a slight shift as treatments got longer. The slight change in phase angle can show, that the nature of electrical conductivity did not change under the various treatments. The increase of impedance magnitude can be caused by increasing viscosity of meat tissue in consequence of water loss.

The shape of impedance spectrum – the slope of curves at low frequencies and the values at higher frequencies - depends on the treatments. The ratio of impedance magnitude at 100 Hz and 1000Hz to impedance magnitude at 100 kHz and 1000 kHz can be used for characterization of changes caused by various treatments.

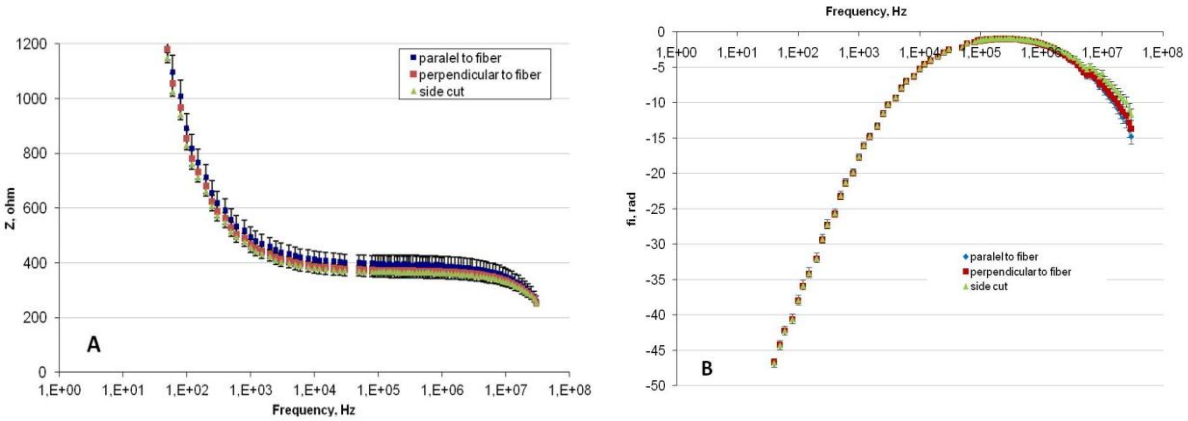

Figure 1: The impedance magnitude (A) and phase angle (B) spectra of the treated meat (300 MPa HHP for 5 min. and 4,5h sous-vide cooked at 60°C): average of measured values in 3 directions with standard deviation.

The water loss can be used for describing the effect of various treatments. The ratio of impedance magnitudes at two different frequencies (Figure 2.) – e.g. Z_{100Hz} / $Z_{1000kHz}$ and Z_{1000Hz} / Z_{100kHz} – slightly increases or practically constant as the water loss increases from 0 up to 10-20 %, and decrease as the water loss continues to increase. The results suggest that there is a virtual border around the sample's 20% water loss value. Below this bordervalue the impedance ratio of the samples showed a slight increase but reaching the 20% and above it showed more important decrease at 100Hz / 1000kHz.

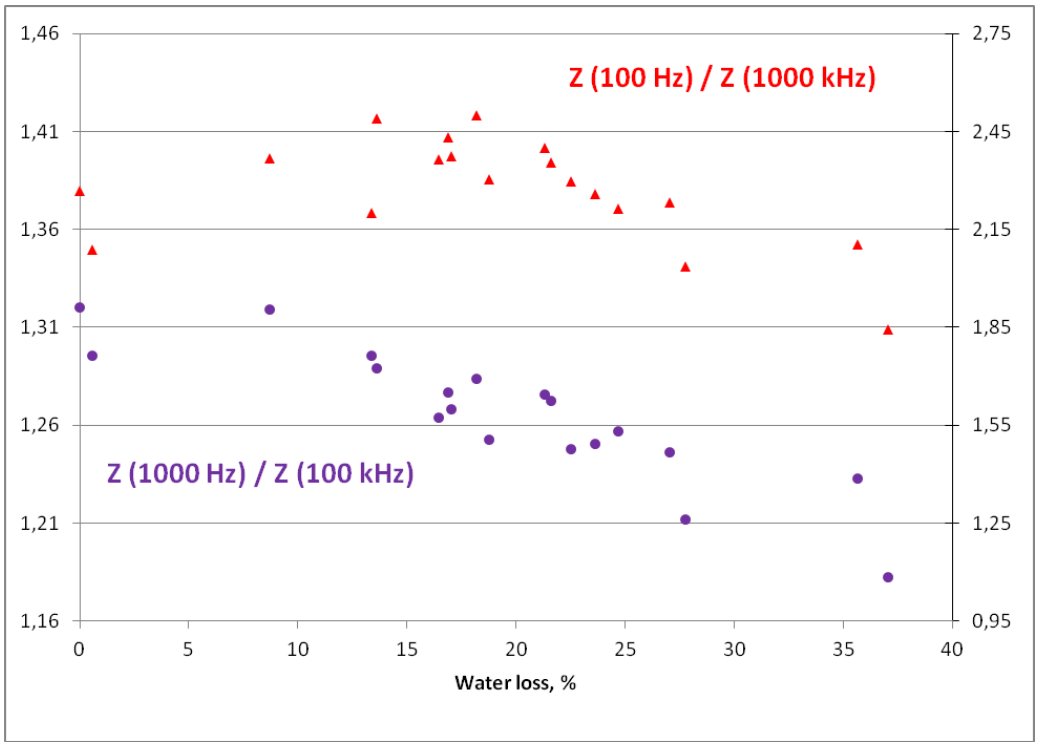

Figure 2: The ratio of imedance magnitudes at various frequencies in the function of water loss

The texture analysis showed a simillar tendency in the function of water loss of samples. In comparison with texture measurement we have found the same border at 20% water loss. (Fig. 3.)

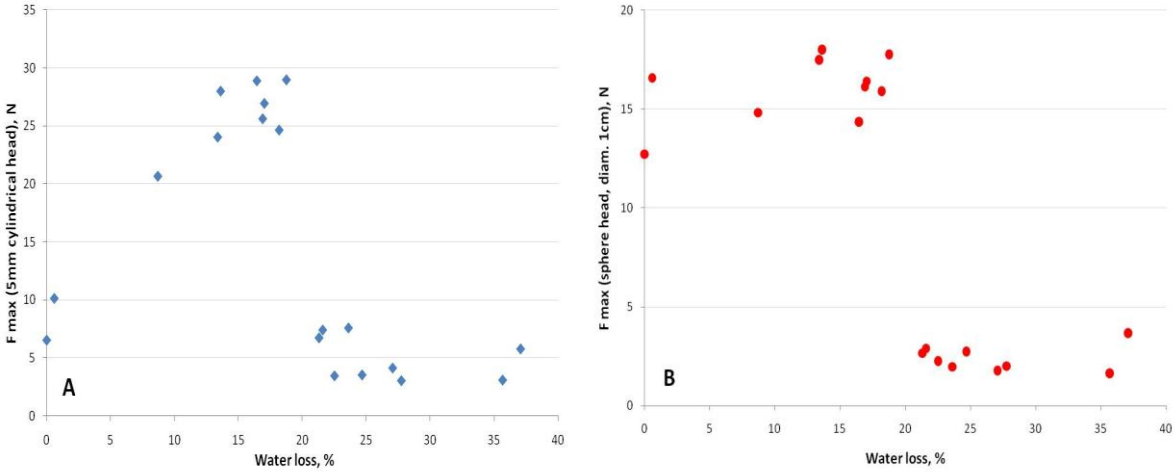

Figure 3: Water loss plotted against the maximal force determined by two different penetrating heads (A: cylandrical; B:sphere)

4. CONCLUSION

We suppose that water loss during meat treatment has an important role in the quality of the product. To determine the effect of different treatments: the calculation of the ratios of impedance magnitude at two frequencies is an adequate method to separate the different treated samples. The longer and „heavier" the curing the bigger the water loss was and in relation to this the impedance value was higher. Further measurements and studies are planned to investigate the possibility of use of impedance parameters in quality assessement of sous-vide and HHP processed meats.

ACKNOWLEDGEMENTS: *This research has been supported by the TÁMOP 4.2.1/B/09/1/KMR/-2010-0005 program of the National Development Agency.*

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INNOCOACHING©: THE NEED FOR COACHING IN THE R&D SECTOR

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SUMMARY

InnoCoaching is a special innovative program for researchers developed by Innogrant Consulting and Coach Academy. The main purpose of the program is to improve researchers' performance and efficiency priorizing for knowledge communication and research funding. The business coach helps researchers in utilization of their opportunities during the coaching process by professional methods.The main difficulty in the field of R&D related research services comes from the coordination of two different approaches: business and researcher interests. Their common purpose is to create something „NEW", founding non-existing technologies and knowledge for the benefits of mankind. InnoCoaching is specially designed for the particular needs of scientific thought. It greatly helps in facilitating professional and personal development to the point of individual growth and improved researcher performance.

FROM IDEA TO MARKET

The mission of Innogrant Consulting is to support strengthening R&D and innovational activities in Hungary and Central Europe bridging between innovation centered organizations and facilitating the implementation of innovative projects with highly added value. By our renewed service portfolio Innogrant is ready to assist our partners during the entire development process from the elaboration of plans to the market launch phase.

Regarding to the „european paradoxon" there is a gap between research and market in the European Union. It means that R&D and innovation activity is world-class but not bringing direct economic results because the innovations hardly reach the market.

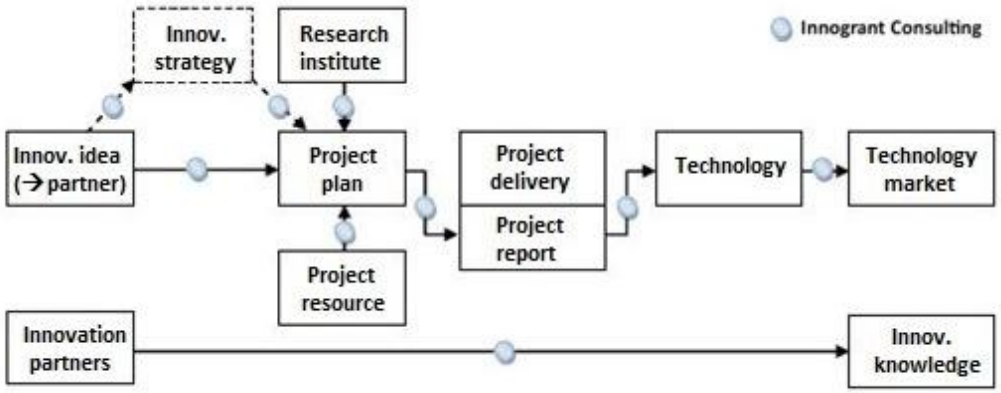

Figure 1: Value chain in R & D and innovation

Today's global economic environment became much complex and demands more conformation and flexibility than ever. These challenges suppose closer cooperation between researches and the business sector. There is a need for problem-solving market-oriented researcher outlook for the implementation of these changes.

Cooperation between the academy and business and the enhancement of their performance and efficiency is emphasized economical interest. InnoCoaching program supports researchers to explore and develop their abilities also on the field of knowledge communication and research funding. There are remarkable results of coaching by the aspect of productivity and output based on the studies. Satisfaction and teamwork gradually improves due to InnoCoaching program. The coaching activity in multinational market environment is proven to be effective with high return. InnoCoaching program has been developed based on the specialty of the scientific purposes and the experience what can boost the efficiency of researchers.

InnoCoaching is beneficial for any researcher regardless of professional experience and research field.

Table 1: Main features of the researcher career

	junior researcher	**senior researcher**	**leader researcher**	**„super researcher"**
field of research	immature, plastic	mature, well-defined	growing fields	existing fields in new context
research results	remarkable	significant	cutting-edge results	revolutionary results
role in research team	execution	responsible team member	team leader	team builder
role in business	employee	employee	owner/leader of succesful enterprises	owner/leader of succesful enterprises on global market

Source: InnoCoaching Marketing Document ©

InnoCoaching program has been developed for both individuals and groups by the differences between career phases of the researchers. The emphasized viewpoint of this program is to ensure professionality. Innogrant Consulting and Coach Academy set up new methodology of coaching resulting InnoCoaching program. The outstanding quality of the InnoCoaching is ensured by significant professional experience of the Coach Academy and international educational material with experienced colleagues.

THE INNOCOACHING PROGRAM

The coaching process reveals the underlying causes and effects considering the determined baseline of the researcher or researcher team by identification of durable and usable solutions. The aim of the program is to improve researcher performance and efficiency. InnoCoaching process relies on the researchers' personal abilities developing them to provide opportunity for continuous self-development.

The main benefit of the team coaching is the utilization of the synergies between the team members. Individual coaching is personalized supporting-developing process what flexibly suits to the personal needs of the researcher. Coachees set off from personal and team problem or situation and reach general level by inductive approaching to foster the change of their perspectives.

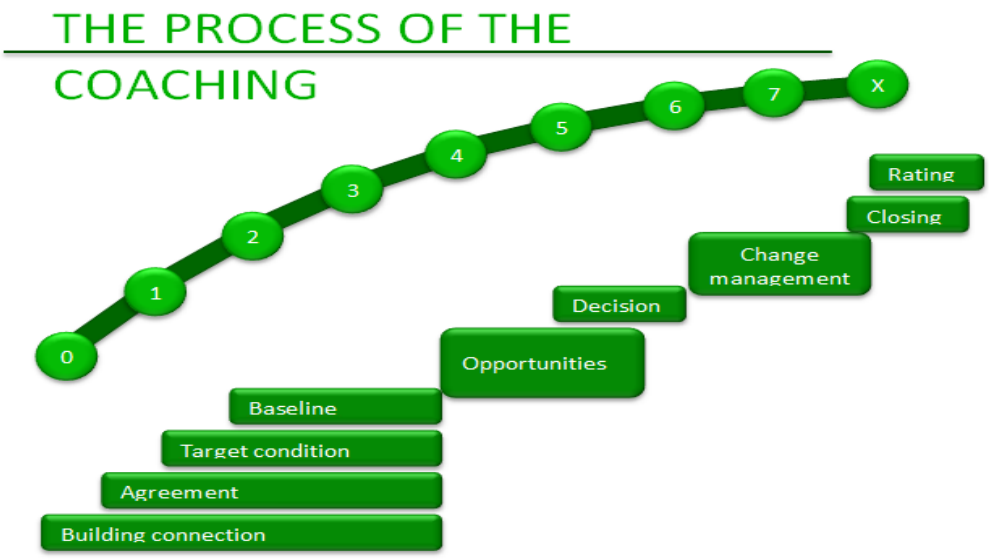

Figure 2: The process of the coaching

The most important element of the support development is the coach's honest, attentive and empathic behavior what grounds the athmosphere of trust between the coach and the researcher. This connection provides a framework for the effective developing work. Coach can be intermediate between the business approach and the researcher society, promoting the harmony of both interests. Researchers often focus only on specific research details which can cause conflict to the market interests. The cooperation of the ademy and business sector is essential to establish a competitive knowledge-based economy.

Researcher's personal performance is a key element of the R&D process. InnoCoaching program's direct support indicates indirect output improvement for the researcher sector supporting sustainable social and economic growth.

THE SUPPORT

InnoCoaching program is focusing on the development of human resources. Working in research centers fosters the performance growth of the R&D and innovational sector. During the program the coach can support the researchers in many versatile ways. InnoCoaching helps focusing on real problems by using targeted questions and ensuring objective feedbacks.

The coach can help especially in the following processes:
Baseline determination:

- helps in visualization of the objectives

Exploration of opportunities:

- helps in development of the researcher's abilities
- contributing the proper use of knowledge depending on the context
- increases awareness
- helps seeing the real alternatives

Decision:

- increases decisiveness
- increases personal responsibility

Implementing change:

- stimulate to act
- maintain motivation
- helps in mobilizing energy reserves

The InnoCoaching program supports premium researchers to achieve their goals and create significant value in international projects.

DEVELOPMENT AND HUMAN CLINICAL STUDY OF VEGETABLE CREAMS HAVING HIGH ANTIOXIDANT CONTENT

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Within the framework of a consortium cooperation („Development of high added value vegetable-based product lines and processing technologies in the interest of the healthy nutrition - USOK2009" project) we have developed a vegetable cream product range spreadable for toast and bread, based on traditional recipes. Basic vegetable ingredients of this product range are tomato, paprika (bell pepper), eggplant and onion.

From phytonutrient content point of view the most promising item of the product range is the vegetable cream with paprika. The daily portion of this product (100 g) contains significant amount of bioactive carotenoids (lycopene, β-carotene, zeaxanthin) which is comparable with RDA's of these components; as well as that contains considerable amount of vitamin E and C. In possession of an ethical committee permit we have performed a human clinical study in order to determine among others whether the human body would be more resistant against oxidative stress after 21 days of regular consumption of 100 g/day vegetable cream.

The study has been an interventional, double blind, randomized study and subjects consumed either the experimental sample, or a control sample having 5 % experimental sample content. 60 healthy persons have been selected for the study; before and after the intervention fasting blood sampling has been performed for the subjects. Before the test a 3days dietary survey has been performed as well. Beyond the regular biochemical analyses we examined the serum lycopene and β-carotene levels, the activities and gene expression of enzymes playing role in the antioxidant defense system as well as immune and genetic toxicology tests has been done from peripheral lymphocites.

Based on the results of our studies we can state that beyond the significant increase of serum lycopene and β-carotene level the most remarkable effect of consumption of vegetable cream is the strenghtening of protection against damages caused by free radicals - which has been detectable by the modified Comet-assay.

USE OF IRRADIATION TO PROVIDE WIDER SELECTION OF FOODS FOR IMMUNO-COMPROMISED PATIENTS

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SUMMARY
Patients undergoing certain therapies and persons in immunsuppressive status are vulnerable to all sorts of infectious complications. Studies have suggested that sterile food is not required for all types of immune-suppressed patients' therapies, but low microbial foods can also be used.The aim of the IAEA coordinated research project is to use irradiation to provide wider selection of safe, nutritionally and organoleptically adequate foods for immuno-compromised patients. Food items has been selected to meet both the dietary recommendations of medical experts and requirements of patients. Sensorially acceptable maximal radiation doses for fresh, pre-cut, prepared fruits and vegetables, dairy products and desserts have been established. Examination of changes of the "native" microflora, nutritional and organoleptic properties of irradiated food items has been performed.

1. INTRODUCTION

Patients undergoing certain therapies and persons in immunsuppressive status are vulnerable to all sorts of infectious complications from any viral or bacterial sources. To prevent such infectious complications, patients are placed in a sterile environment. They are given antibiotics to sterilize gastrointestinal tract and are put on a sterile diet. Most canned foods are sterile, and are thus acceptable for these patients. Some foods – bread and bread products, cereals, pastas, condiments and desserts - however, do not withstand autoclaving. The changes due to heat are to such a degree that patients either reject the food because of appearance, or they won't eat it because the flavor and texture have changed. Irradiation has been successfully used for sterilization of such foods for hospital patients (Aker, 1984). Studies have suggested that sterile food is not required for all types of immune-suppressed patients' therapies, but low microbial foods can also be used (Aker and Cheney, 1983). To reduce the risk of infection of people with lowered immunity, several preventive measures have been adopted in hospitals (Alothman, 2005; van Tiel et al., 2007). The measures include protective isolation, antibiotic prophylaxis and the use of low-bacterial diets.

The overall objective of the IAEA co-ordinated project is to use irradiation to provide wider selection of safe, nutritionally and organoleptically adequate foods for immuno-compromised patients. Food products for examination were selected according to the survey of Hungarian institutional practices for dietary restrictions for immunosuppressed patients carried out by the National Institute for Food and Nutrition Science (OÉTI). It was understood that consumption of some desserts or sweets can enhance the quality of life of these patients.

The aim of our present studies was to determine the radiation doses provide microbiological safety of dessert products without diminishing the quality/nutritional/sensory parameters of fruit purees, chestnut puree and sponge cake.

2. MATERIALS AND METHODS

2.1. Dessert food items for irradiation and microbiological / organoleptic examination

Selection of food items for investigation has been carried out according to the requests of the health-care institutions.

Raspberry puree:

Frozen raspberry were purchased from the local market. After thawing, fruit puree was prepared with a blender. Approx. 50 g portions of raspberry puree were placed aerobically in

covered plastic (PE) containers. Samples were frozen again and kept at -18 °C until radiation treatment.

Sweet chestnut puree:

Frozen sweet chestnut puree was purchased from the local market. After thawing, 50 g portions of puree were placed aerobically in covered plastic (PE) containers. Samples were frozen again and kept at -18 °C until radiation treatment.

Sponge cake:

Fresh sponge-cakes were purchased from the local market. Cakes were sliced into approx. 50 g pieces and placed in covered plastic containers. Samples were frozen and kept at -18 °C until radiation treatment.

2.2. Radiation treatment

Packaged products were irradiated frozen with doses of 1 kGy, 2 kGy and 3 kGy at a dose rate of 5.53 kGy/h by a ^{60}Co irradiator at AGROSTER Co. Ltd, Budapest.

2.3. Storage of food items

Irradiated and unirradiated control samples were stored at -18 °C for 6-7 days.

2.4. Microbiological analysis

Microbiological analysis was carried out periodically in triplicates.

Total aerobic plate counts were determined on Plate Count Agar (MERCK 1.05463), plates were incubated at 30 °C for 48 h. **Aerobic spore count**: aliquots of sample homogenates were heated at 80 °C for 10 min and pour plate with Plate Count Agar (MERCK 1.05463). Plates were incubated at 37 °C for 48 hours. **Anaerobic spore count**: aliquots of sample homogenates were heated at 80 °C for 10 min and pour plate with Reinforced Clostridial Agar (MERCK 1.05410). Plates were incubated under anaerobic conditions at 37 °C for 96 hours.

Total coliforms were determined with spread plate on Chromocult Coliform Agar (MERCK 1.10426), incubation at 37 °C for 24 hr. **Yeast and mould count**: surface spreading on Dichloran Rose-bengal Chloramphenicol Agar (MERCK 1.00467), incubation at 25 °C for 3-5 days. **Lactic acid bacteria (LAB)**: pour plating with double layer of MRS agar (Lactobacillus agar acc. to DE MAN, ROGOSA and SHARPE, MERCK 1.10660), incubation at 30°C for 48 hr. ***Staphylococcus aureus* count**: according to EN ISO 6888-1:1999; ***Salmonella* spp**: presence/absence-test according to EN ISO 6579:2002; ***Listeria monocytogenes:*** presence/absence-test according to EN ISO 11290-1:2004, detection method.

2.5. Statistical analysis of experimental data

One-way ANOVA analysis was carried out by using PAST ver. 2.15 software to determine significant differences between treatments.

2.6. Estimation of sensorically acceptable radiation dose of raspberry-chestnut puree-sponge cake dessert

Dessert has been prepared with sweet chestnut puree and raspberry puree on top of a piece of sponge-cake, packaged in covered plastic containers. Frozen samples were irradiated with doses of 2 kGy and 3 kGy at AGROSTER Co. Ltd, Budapest.

After thawing at room temperature, unirradiated control and irradiated batches of chestnut-raspberry dessert were analysed sensorically by a sensory panel of 19 people directly after the treatment on the basis of hedonic scores on colour, odour, taste and texture ranging from score (9) as excellent to score (1) as non-marketable.

3. RESULTS AND DISCUSSION

3.1. Microbiological evaluation

Raspberry puree

The radiation treatment with **3 kGy** dose reduced the relatively low initial **total aerobic plate counts** of frozen raspberry puree (initial pH 3.2±0.2) count below detection limit.

Yeasts formed the dominant part of the microflora of raspberry puree. They were relatively sensitive to low dose irradiation, probably due to the acidic conditions of raspberry. Yeast counts of 3 kGy irradiated raspberry puree samples were below detection limit (<50 CFU/g). **Moulds** were present in low initial numbers in raspberry puree and their numbers on 3 kGy irradiated samples were below detection limit during storage.

Lactic acid bacteria were in low numbers present in frozen raspberry puree. **Total coliforms** were present in <15 CFU/g in all irradiated and control frozen raspberry puree samples. The **number of aerobic and anaerobic spore formers** was below detection limit in all irradiated and control raspberry puree samples.

Sweet chestnut puree

The radiation treatment with **3 kGy** dose reduced the initial **total aerobic plate count** by about two log-cycles.

Yeasts formed a large part of the microflora of sweet chestnut puree. Yeast counts of 3 kGy irradiated sweet chestnut puree samples were below or around microbiological criteria (<50 CFU/g) suggested by the CRP.

Moulds were present in low initial numbers in sweet chestnut puree and their numbers in 3 kGy irradiated samples were below detection limit during storage.

Total coliforms were detected in control samples in relatively high numbers (~2.6 log CFU/g) showing the lack of good hygiene practices during production of frozen sweet chestnut puree. Radiation doses of ≥2 kGy reduced their numbers below detection limit. The **number of aerobic and anaerobic spore formers** was below detection limit in all irradiated and control samples.

Sponge cake

Radiation treatment with 3 kGy dose reduced the total aerobic plate count sufficiently (<100 CFU/g).

Yeasts were present in sponge cake in numbers about 100 CFU/g. Yeast counts of 3 kGy frozen sponge cake samples were at the level of the microbiological criteria (<50 CFU/g) suggested by the CRP.

Moulds were present in low initial numbers in sponge cake samples and their numbers on 3 kGy irradiated samples were below detection limit during storage.

Total coliforms were not detected in any of the irradiated and control sponge cake samples.

Aerobic spore formers were detected in unirradiated control samples in low numbers, radiation doses of ≥2 kGy reduced their numbers below detection limit.

The **number of anaerobic spore formers** was below detection limit in all irradiated and control frozen sponge cake samples.

Staphylococcus aureus* counts** were <15 CFU/g in all irradiated and control samples, ***Salmonella* spp** and ***L. monocytogenes were not detected in 25 g of samples.

3.2. Estimation of sensorically acceptable radiation dose of raspberry - chestnut puree - sponge cake dessert

Irradiation has been carried out in frozen form to avoid/reduce undesirable oxidative changes in the dessert products.

Sensory testing of raspberry-sweet chestnut puree-sponge cake dessert showed that according to Kramer's rank test, statistically significant differences in organoleptic properties (colour, odour, taste and texture) were determined in 2 kGy and 3kGy irradiated samples compared to the unirradiated controls (Table 1.) However, all samples were acceptable and marketable.

Table 1. Sensory testing of chestnut-raspberry dessert (19 panelists)

Radiation dose (kGy)	Score means								Rank sums			
	Colour		Odour		Taste		Texture		Colour	Odour	Taste	Texture
0	8.32	±0.89	8.05	±0.91	8.37	±1.26	8.05	±1.08	**28.00**	**24.50**	**24.50**	**28.50**
2	6.84	±6.58	5.74	±2.35	6.79	±1.72	7.00	±1.73	44.00	45.00	42.50	42.50
3	6.58	±2.22	5.63	±2.36	6.53	±1.58	6.79	±2.02	42.00	44.50	**47.00**	43.00

* rank sums within the range 30-46 are not significantly different at $\alpha \leq 0.05$ probability level

Fernandes et al. (2011) studied the effect of irradiation on the chemical composition of Portugeese chestnut fruit. Their results indicated that application of gamma irradiation with ≤ 3 kGy doses did not affect the nutritional and chemical quality (sugars, fatty acids, tocopherols) of chestnut fruits.

4. CONCLUSIONS

Irradiation of frozen raspberry puree, sweet chestnut puree and sponge cake dessert with 3 kGy dose can provide appropriate low microbial counts. Samples remain microbiologically safe during 7 days of frozen (-18 °C) storage. Sensory testing by healthy adults showed that although statistically significant differences in organoleptic properties (colour, odour, taste and texture) were determined in irradiated (2 and 3 kGy) desserts, all samples were acceptable.

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MODIFICATIONS OF PHYSICAL PROPERTIES OF COCONUT OIL AND ANYHIDROUS MILK FAT AS A RESULT OF BLENDING

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SUMMARY
The role of fats in food technology is mainly to develop the desired consistency. The simplest way to reach this goal is blending different fats. The aim of our work was to study the physical properties of blends of coconut oil and anhydrous milk fat. Pure fats and their 25-75%, 50-50%, 75-25% blends were investigated. Melting profile and isotherm crystallization were measured by pNMR. Rheological characteristics were analyzed by rheometer both in rotation and oscillation mode. Possible applications of the blends were established.

1. INTRODUCTION

Coconut oil is one of the most widely used fats in food industry. Due to its melting and crystallization characteristics margarine and shortening production as well as confectionary industry consider coconut oil as a basic material of product formulation. Since these kinds of products consist of other fat materials it is interesting to study the counter relationship of coconut oil and other fats if they are blended. In our study coconut oil and anhydrous milk fat (AMF) were blended in different ratios and the modification of the most important physical properties was studied. These are: melting profile and isotherm crystallization as well as some rheological parameters such as viscosity. Earlier studies indicated that the modification of these parameters should be highly affected by the restricted miscibility of coconut oil and AMF.

2. MATERIALS AND METHODS

2.1. Materials

Anhydrous milk fat was gathered from local confectionary factory. Origin the coconut oil was commercial wholesaler (Barco). 500 cm^3 of the materials was heated up to 80 °C in order to eliminate crystal structure. Pure fat samples and 25-75%, 50%-50 % and 75-25% blends of AMF and Coconut oil were prepared in liquid state in volumetric flasks and then cooled to 5 °C and kept in refrigerator until measurements were conducted.

2.2. Methods

Melting profile of the fats was established by the solid fat content curves (SFC) by means of pNMR apparatus (Bruker Minispec 120, Bruker, Germany) following the AOAC method. The samples were heated to 80 °C and held for 15 min in order to eliminate crystal memory. All samples were kept at 60 °C for 15 min., cooled down to 0 °C and maintained at this temperature for 60 min. Finally, before measurements, samples were held at the temperature of the measurements for 30 - 35 min. Temperatures were: 10 °C, 20 °C, 30 °C, 40 °C, and 50 °C. Three parallel measurements were done and average values were reported.

Isotherm crystallization was detected by measuring solid fat content with pNMR (Bruker Minispec 120) apparatus. Measurement based on method reported by CAMPOS and co-workers (2010) were modified as follows: Samples were heated up to 80 °C and kept there for 15 minutes. The completely liquid samples were adjusted in NMR tubes and put into a 5 °C

thermostat. Measurements were done every 5 minutes for 6 hours. Three parallel measurements had been done and average values were reported.

For rheological measurements Anton Paar Physica MCR 301 rheometer was applied for rotation and oscillation mode. Measurements were performed at 50°C. Cone-plate arrangement was chosen for oscillation conditions.

3. RESULTS

Solid fat content of the samples is shown in Figure 1.

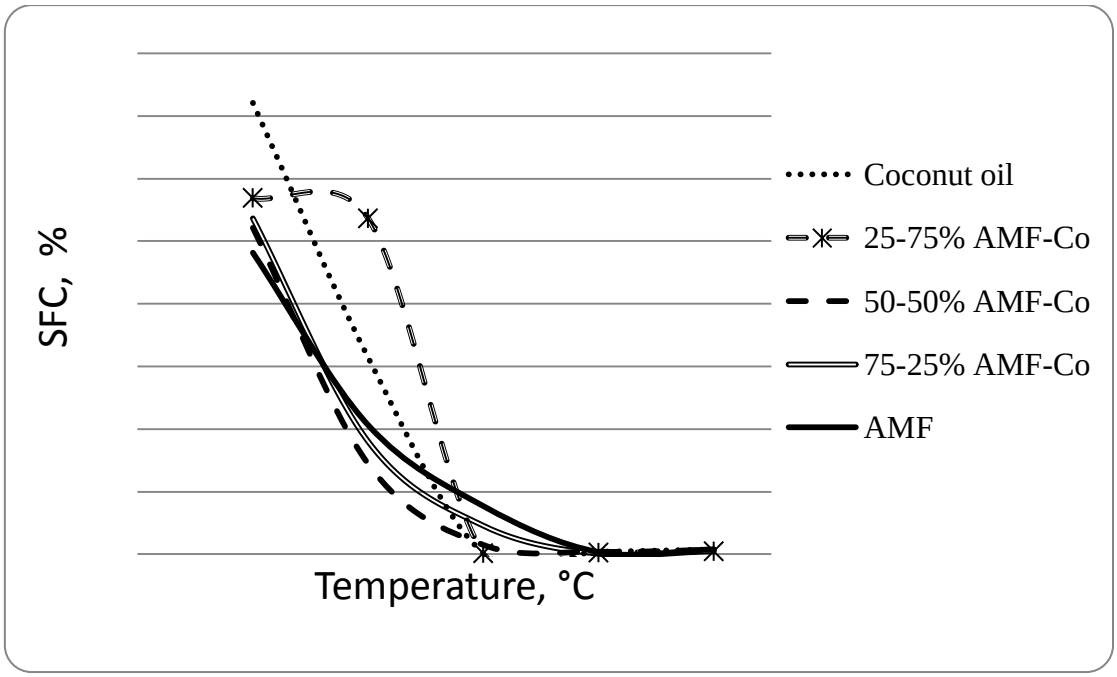

Figure 1: Solid fat content of AMF, Coconut oil and their blend

From the figure it can be seen that coconut oil had the highest SFC value at low temperature. All samples melted rather fast. Fats blended in 50-50% ratio performed a characteristic plateau in SFC curve between 10°C and 20°C. At higher temperatures AMF and 50-50%, 75-25% (AMF-Co) contained more solids than pure coconut oil and 25-75% samples.

Results of isotherm crystallization of the samples are demonstrated in Figure 2.

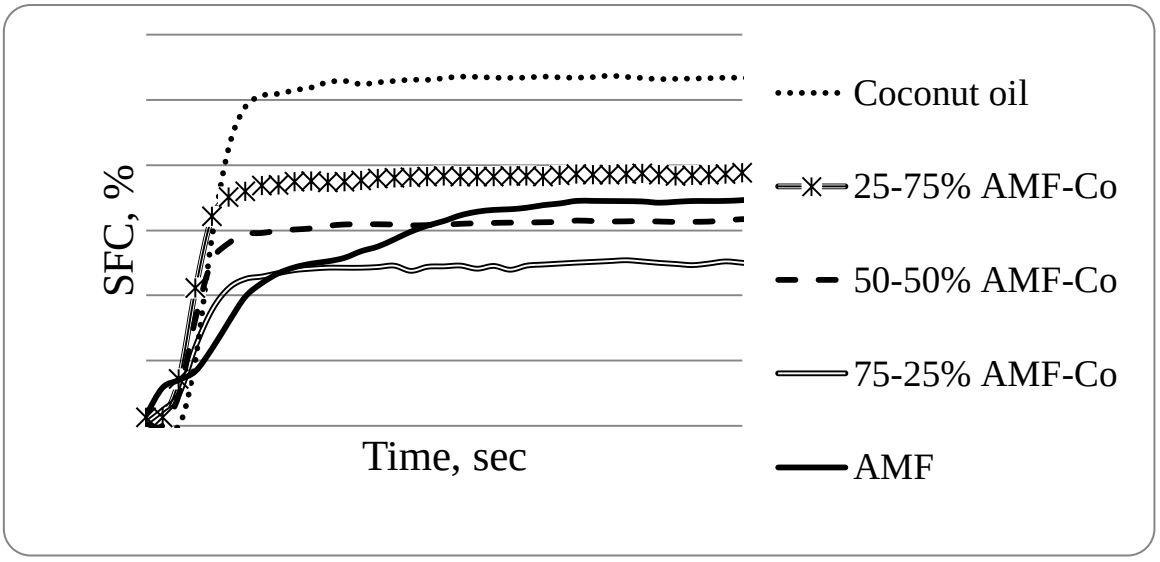

Figure 2: Crystallization of AMF, Coconut oil and their blend

Crystallization curves show that approximately within 50 min. fats solidified almost completely. It is also clear that AMF solidified in a two step mechanism and the other samples show different phenomena. Curves of coconut oil and the blends run similarly, and the equilibrium SFC decreased by the increasing amount of AMF in the blend. Equilibrium SFC of AMF was closer to the blend of 25-75% AMF-Coconut oil and was higher than the other blends'.

Of the rheological investigations, results of viscosity obtained by rotation and oscillation measurements are shown in Table 1.

Table 1: Complex viscosity of AMF, Coconut oil and their blend

	Shear rate/angular frequency (1/s)	Complex viscosity (Pas)				
		Coconut oil	25-75% AMF-Co	50-50% AMF-Co	75-25% AMF-Co	AMF
ROT.	0,1	0,143	0,069	0,166	0,120	0,278
	1	0,029	0,020	0,034	0,025	0,034
	10	0,021	0,022	0,022	0,023	0,025
OSC.	1	0,021	0,022	0,024	0,025	0,064
	10	0,045	0,035	0,039	0,049	0,051
	100	0,430	0,315	0,348	0,464	0,409
	600	2,300	1,570	1,853	2,447	2,140

From the table it is clear that while in rotation mode viscosity decreased along increasing shear rate, so increasing angular frequency caused higher complex viscosity in oscillation mode. Individual values are different in the low shear rate interval but became closer if the shear rate is higher. In contrast the increasing angular frequency resulted smaller complex viscosity values at 25-75% and 50-50% blends of AMF and coconut oil.

4. CONCLUSIONS

In our investigations coconut oil proved to be the hardest fat at low temperature, but melted rather fast. At 30°C the coconut oil samples melted completely. AMF was less hard at low temperatures but above 25°C contained more solids than the coconut oil. This finding indicates a eutectic phenomenon that is possibly characteristic to these fats. As a conclusion it can be stated that while the lower temperature range AMF is the solvent of the coconut oil, at higher temperatures the effect is inverse. From the SFC curves the maximum miscibility is estimated to be 25-75 % (AMF: Coconut oil)

The eutectic phenomenon was not clear in case of isotherm crystallization at 5°C. Coconut oil and their blends show a similar profile and differed from the shape of the crystallization of AMF. We could conclude from this that the effect of AMF on the crystallization mechanism of the coconut is not strong.

Rheological measurements proved that the investigated fats became similar under high shear effect. All the fat samples showed shear thinning features. Oscillation effect caused an increase in complex viscosity but the effect was anomalistic.

As a final conclusion we could state that AMF should be use to tenderize the hard coconut oil at low temperatures but the effect is limited by the restricted miscibility of these fats.

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INSPECTION OF MARBLING, SEUROP QUALITY LEVEL AND PORK LOIN QUALITY CHARACTERISTICS

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SUMMARY

In our study we have examined the connection between the marbling grade and the quality level of pork loin and further the possible methods that are used to grade the marbling characteristic. During the measurements we have conducted on the one hand instrumental measurements for colour, texture, pH, water holding capacity and on the other sensory evaluation for hardness, juiciness, taste, fatness and overall impression. The marbling quality was defined by a brain trust on a scale of 1-5.

Based on our study we have found that the quality level of pork loin corresponds with their marbling, as the quality level decreases (S→O) the level of marbling rises. We have also established the methods that are suitable to establish and separate quality levels and marbling; these are the objective colour and texture measurements, especially the measurement for red colouring (a).*

1. INTRODUCTION

The qualification of pork has changed significantly in the past century. Fat and bone yield figures were replaced with meat yield target numbers, measurement methods have greatly improved and been simplified as well. In Europe, the most widespread measurement system is the SEUROP for meat grading and to determine the sales price of pork. Although this is suitable to grade meat content it is not known whether it is able to provide ample information for the consumers on sensory value, i.e. enjoyment value (Szűcs, 2002).

Marbling of meat (the fine intramuscular fat tissue covering fibre bundles) is used to define the tenderness and juiciness of meat chops (steaks). Thus the evaluation of marbling, its extent and distribution is a basic characteristic used to define meat quality.

It is a well-known fact that in Anglo-Saxon countries as well as in Japan the marbling of beef is of significant importance but as we are going to demonstrate it is well worth exploring this topic in more depth with pork as well, either for sales promotion (brand building) or for a select premium customer group (William, 2002).

Our aim was to define the connection between the marbling grade and other meat characteristics. That is why we examined the connection between the SEUROP grading method and measured meat characteristics and between marbling and the SEUROP grades.

2. MATERIALS AND METHODS

We used S, E, U, R and O graded pig carcasses from the same site, the pigs were of the same breed (large white meat swine types), same age and sex and reared and fed under the same conditions. Note that all these parameters have an impact on the marbling (Carrasco et al., 2009). Carcasses were boned by the same method to obtain boned pork loin (*longissimus dorsi*), cut them into 4 same-sized parts and finally made 2-cm-thick slices.

Afterpreparation we have conducted the following measurements and statistics:

- ***Colour measurement:*** MINOLTA CR-200 chroma meter in the CIELAB system. **Measured parameters: lightness (L*), red (+a*), green (-a*), yellow (+b*) and blue (-**b*) colours;
- ***Water holding capacity:*** pressing test, Grau und Hamm method,
- ***Moisture and dry matter content:*** according to standard MSZ ISO 1442:2000,
- ***Sensory evaluation:*** we roasted the meat to 72 °C core temperature and a panel of 5 trained assessors carried out the sensory evaluation. Besides the descriptive evaluation we applied a scale of 1-10 (10: best quality),
- ***Texture (transversal resistance)*** was measured with the TA-XT2i (Stable Micro Systems) device using a Warner-Bratzler probe. We roasted the meat to 72 °C core temperature, dried it with filter paper and cooled to room temperature (20 °C). We cut cylinders with 11 mm height perpendicular to the fibres and transversal resistance was measured perpendicular to the fibres,
- ***Weight loss at roasting*** was measured by weighing the slices after roasting;
- ***pH- measurement:*** we used a WTW 330i pH-meter with an electrode probe
- ***Marbling grading:*** visual inspection by brain trust on a grade of 1-5 (1: light marbling, 5: heavy marbling).

The measurements were carried out at the National Meat Research Institute and the, Department of Refrigeration and Livestock' Products Technology, Faculty of Food Science of Corvinus University of Budapest. Data from the two laboratories were handled together.

During the study the results from the same category (SEUROP) meat were summarised and averaged based on the pieces and slices.

During the data analysis we examined and re-checked the extreme and missing values then we examined the connection between quality (SEUROP) levels (1-4) and slices (1-3). We used a two-way analysis of variance to establish the expected value of the population taking into consideration experimental errors. Processed statistical data were compared with SEUROP levels and marbling grades. To establish correlation we used Pearson correlation coefficient (R^2), the significance level was 95%.

We used MS Excel and Visual Basic to data analysis.

3. RESULTS

While processing the statistical information occurrence of extreme values remained below 1% and we eliminated their interference. 0,005% of the data was missing, which we handled with averaging relevant rows and columns.

We have found that there were no significant differences between the same SEURO categories and between the slices of the same samples. However, we found notable alteration between the different SEURO categories.

We used a two-way analysis of variance to establish that the error rate, with the exception of roast weight loss, was low (p=max 0.057). Some examples are shown in tables 1. and 2.

L* and b* values of colour measurement remained unchanged with S, E, U categories and with R and O categories began to rise slightly. The a* level increased in correlation with the quality level (R^2=0.66).

Water holding capacity and dry-matter content was independent of the quality level thus water holding capacity and dry-matter content cannot be used to establish quality level. Water holding capacity depends on protein and water bond (Huff-Lonergan, 2010).

The scores from the sensory evaluation decreased as the fat content, that is used in grading, increased.

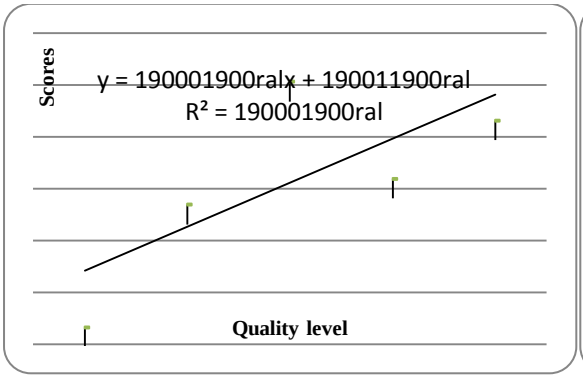

Figure 1: Connection between marbling and quality level

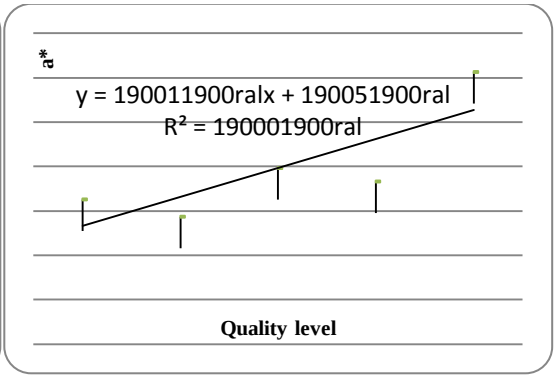

Figure 2: Connection between a* value and quality level

With our tests and the evaluation of the statistical data we have selected the characteristics that corresponded best with the marbling quality of the samples. The following charts display some examples of our results (Figures 3-5.).

We have found that there was no connection between marbling and L* and b* values. The value of a* increased with marbling (R^2=0.73).

Water holding capacity rose until marbling grade reached 2.5 then decreased (larger fat content reduced water holding capacity).

Dry matter content increased linearly (R^2=0.77) with marbling. The spread fat contain causes higher dry matter content.

Sensory evaluation values decreased slightly as marbling grades went up. This was obviously thanks to the higher fat content. However is to be noted that the fatness quality did not change even though the fat content of the samples were different. This was due to the finer spreaddistribution of fat content in the meats with a higher marbling (Brooks et al., 2000).

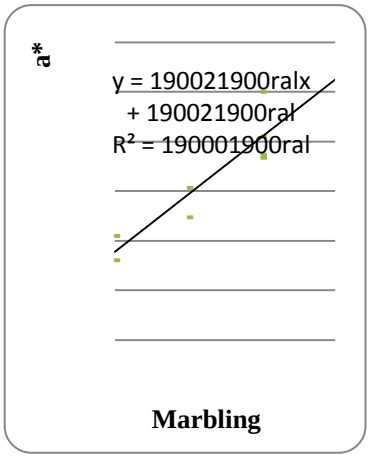

Figure 3: Connection between a* value and marbling

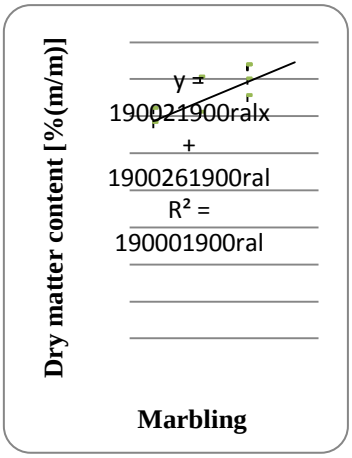

Figure 4: Connection between dry matter content and marbling

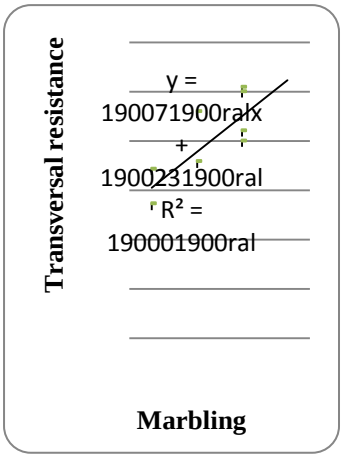

Figure 5: Connection between transversal resistance and marbling

4. CONCLUSIONS, SUGGESTIONS

In our study of pork loin we have found correspondence between SEUROP quality levels and the a* value of the colour measurement. Additionally, we have discovered connections to marbling as well. Based on the correspondence, marbling is related to the a* value, dry matter content and transversal resistance. From the measurement methods, measuring of a* value could be used in the future to categorise pork on the large scale in industrial environment.

More in depth research and data analysis is required to establish the exact locations on the pork loin and slices where the measurements are to be carried out.

The results of our colour measurements shed light on the fact that objective computerized image recording and processing can be used to estimate the grade of marbling. Our result can be used to work out the method for this grading model.

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EFFECT OF APPLE POMACE ON THE TEXTURE AND RHEOLOGICAL PROPERTIES OF BAKERY JAMS

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SUMMARY

Nowadays utilisation of by-products (grape and apple pomace) has increasing tendency in the food industry. Apple pomace is a by-product of apple juice processing industry and contains parts of peel, core and periplast as residual after pressing.

In present work texture of bakery jams were stabilized by dried and fined-grounded apple pomace powder with high pectin content. As control sample, bakery jam produced with industrial baking-pectin was used. Pectin content of experimental samples was decreased from 100% to 0% while amount of apple pomace powder was increased from 0% up to 100%. Texture of baking-jams was measured by Brookfield LFRA Texture Analyzer and evaluated by texture profile. Rheological properties were determined by Physica MCR 51 rheometer by oscillatory technique using amplitude sweep method. According to the results 50% of industrial pectin could be replaced by dried apple pomace powder.

1. INTRODUCTION

1.1. Apple pomace

Apple pomace, a cheap by-product of apple juice production, is rich in pectin and flavour compounds. It could be used for several applications, such as pectin recovery (Schieber, Hilt, Streker, Endress, Rentschler & Carle, 2003), jam and jelly production (Royer, Madieta, Symoneaux & Jourjon, 2006), enzyme production (Favela-Torres, Volke & Viniegra, 2006), animal feed, organic acid production (Shojaosadati & Babaeipour, 2002), ethanol production as a source of aroma compounds and natural antioxidants (Foo & Lu, 1999).

1.2. Jams and jellies

Jam is a low moisture food prepared by boiling fruit pulp with sugar, pectin, acid and other ingredients (preservative, colouring and flavouring agents (Lal et al., 1998; Barett et al., 2005). Total soluble –solids content of the finished jam should be between 60% and the product should contain at least 45% fruit (CODEX, 2009). Bakery jam is a special industrial jam type which texture must to be shape keeper during baking.

2. MATERIALS AND METHODS

2.1. Materials

Raw materials of bakery jams were the follows:

- apple-, apricot-, sour cherry and plum puree (20-20%)
- black currant concentrate (20%)

Apple pomace was produced at laboratory circumstances from variety ´Idared´ (Orchard of Soroksár, Corvinus University of Budapest). After pressing apple pomace was dried at 80°C until reach 5% wet content. Dried apple pomace was grinded by a laboratory grinder (to 0.2 mm) producing apple pomace powder (AP) for the experiments.
Industrial pectin and preservative were purchased from Pacific Óceán Ltd. (Vác, Hungary).

2.2. Preparation of bakery jams

The preparation of bakery jams was performed according to Table 1. Total soluble – solids content of the finished bakery jams were 61.1±1.0%, and fruit content was 55%.

Table 1: Ingredients of bakery jams

Samples	Fruit content (g)	Sugar (g)	Preservative (g)	Pectin (g)	AP powder (g)	AP content %
Control	550	565	10	13	0	0
AP40	550	565	10	12	8	40
AP50	550	565	10	10	10	50
AP60	550	565	10	8	12	60
AP80	550	565	10	6	14	80
AP100	550	565	10	0	20	100

2.3. Rheological measurements

Rheological measurements were performed using a Physica MCR 51 rheometer (Anton-Paar Hungary Ltd., Veszprém, Hungary). Results were recorded and analyzed using Rheoplus software ver 3.2. (Anton-Paar Hungary Ltd., Veszprém, Hungary).

Oscillation tests were performed with a plate and plate measurement system that consists of a P-PTD200 plate and a PP50/S (plate 50 mm in diameter) measuring bob using 1 mm gap size. The amplitude sweep method was performed at 4°C, at constant angular frequency (10 rad s^{-1}), increasing strain from 0.01 to 200%. Measurements were performed with 5 parallels. Storage (G', Pa) and loss modulus (G", Pa) were recorded in function of shear stress (τ, Pa). Based on the amplitude sweep rheograms initial storage modulus (G_0', Pa), initial loss modulus (G_0", Pa), shear stress at the end of linear viscoelastic range (LVE, Pa), shear stress (τ_C, Pa) and complex viscosity (η^*_C, Pa.s) at crossover point; slope of G' curve (S') between LVE and crossover point were determined.

1. RESULTS

The amplitude sweep rheograms of the different pectin content bakery jams are shown in Figure 1. The shapes of the curves were similar: the initial phase of G ' larger-than-G ", which means that the jams are sufficiently strengthened. Beyond a certain shear stress value the G' and G" values began to decrease, and then crossover occurred. By increasing the proportion of apple pomace G' and G" values have decreased. Above 50% apple pomace content the ratio of the two values also decreased suggesting that a weaker gel has evolved such as in the case of samples contain pectin.

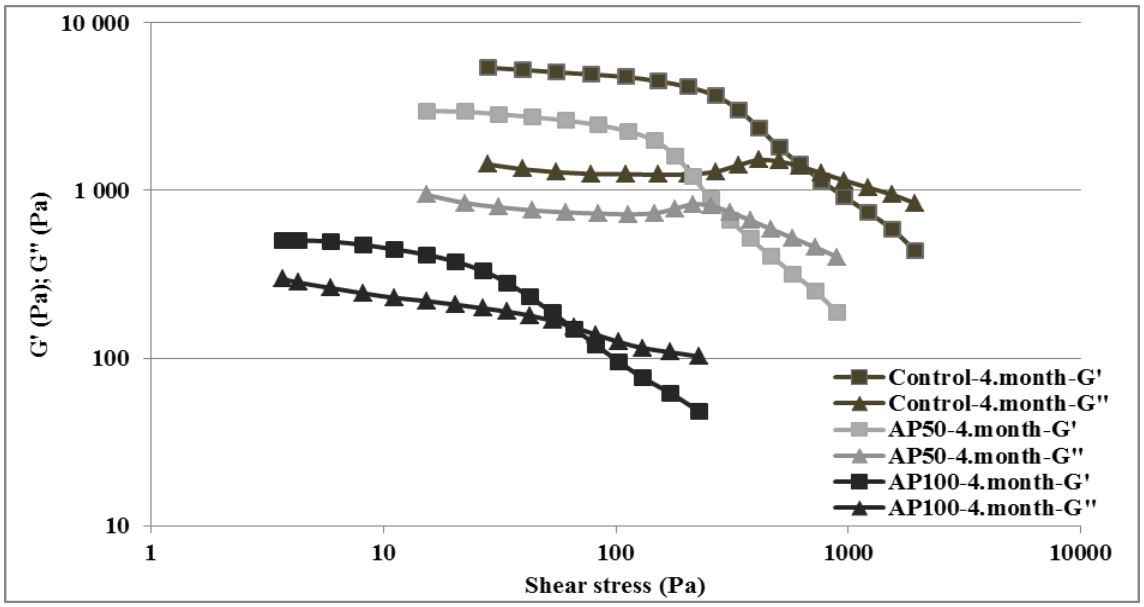

Figure 1: Amplitude sweep rheograms of bakery jams

In Figure 2. G'_0 values of different apple pomace content bakery jams can be seen during 12 month storage. The initial value of G' indicate the solidity at rest: the higher value refers to the harder sample. By increasing the proportion of apple pomace (from 0% up to 100%), G' values decreased, i.e. less flexible, less solid jams managed to produce. However, there was no significant difference between the control and sample AP40.

During storage G' values of the control sample and samples AP40, AP50, AP60, AP80 increased until the 8. month, then slightly decreased. In the case of sample AP100 continouos increasing were observed.

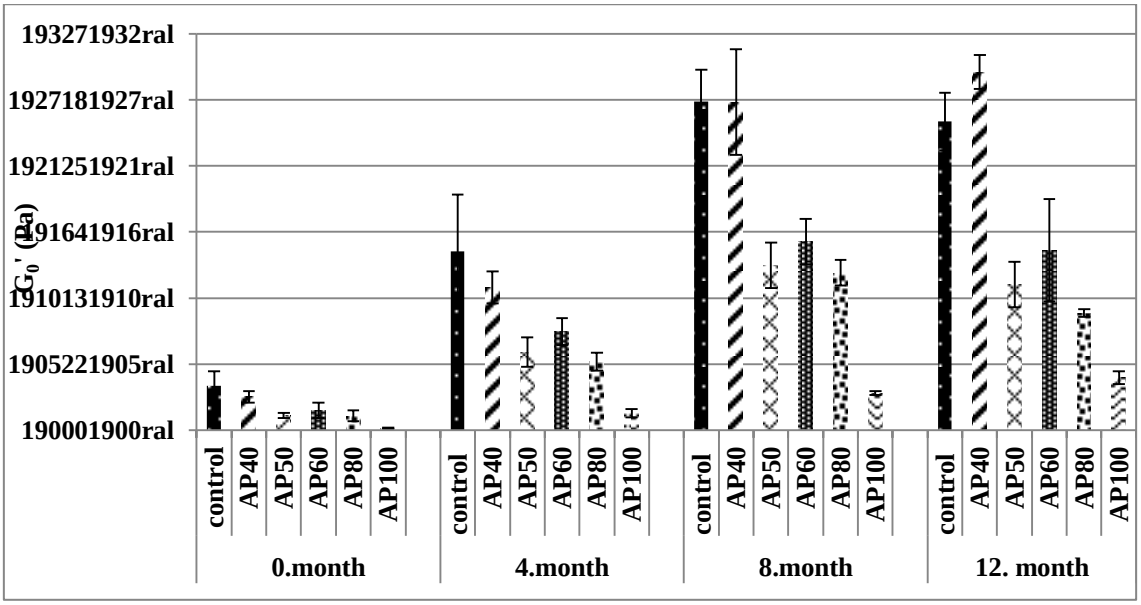

Figure 2: G_0' values of bakery jams

In Figure 3. shear stress values are shown at the crossover point. Crossover indicates the shear stress at which sample turns to viscoelastic liquid. In the case of jams if crossover is higher, the product is more resistant to mechanical influences and more stable.

Control sample showed the highest value and the values were the lowest in the case of sample AP100. During 8 month of the storage there was no significant difference between the control and sample AP40 all the sampling point. During storage in the case of all samples increasing were observed.

By increasing the apple pomace content, crossover decreased, beacuse pomace has lesser water binding capacity as pectin and because of this attribute it can be form weaker gel. It can easier release the bound water and has higher tendency to the syneresis.

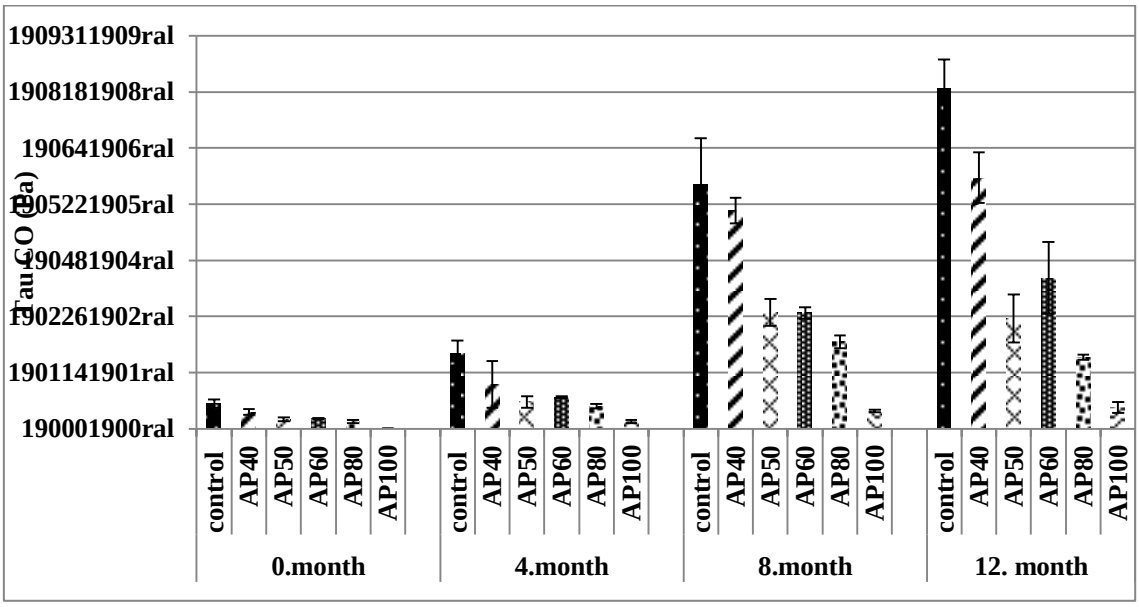

Figure 3: Crossover point of bakery jams during storage

During storage samples became more solid, which could be caused by releasing water or the swelling of hydrofil colloids (e.g. pectin).

Bakery jams which texture was stabilized by apple pomace powder were less solid, but they could be as well stored as control bakery jam which was produced by pectin.

2. CONCLUSION

According to the results a part of industrial pectin could be replaced by dried apple pomace powder. The use of apple pomace as natural texture stabilizer brings us closer to the non-waste fruit processing.

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DILEMMAS OF AGRICULTURAL HIGHER EDUCATION

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SUMMARY

Size and structure of Hungarian as well as international higher education have been considerably changed during the last decades. One of the most important factors of this transition is the series of deep-rooting changes in socio-economic environment. Similar transitions had been only two centuries ago, during the industrial revolution. The situation is paradoxical, because on one hand it is widely accepted, that the educational system in general and system of higher education in particular is a deciding factor of upgrading of international competitiveness, but when decision are formed on allocation of financial resources for development of education –not just in Hungary, but in another member-states of the EU as well- the higher education system is placed at the end of priorities. This tension has been a characteristic feature of the European higher education space, but it has became extremely acute after the 2008 world economic crisis. A considerable part of the "rich" countries spend higher value of monetary resources of higher education, and the lesser developed states decrease their spending on higher education. At the same time, there emerged new demands for traditional agricultural specialists' qualification. There is an increasing necessity of new skills and competences, e.g. in field of mechanisation, robotics, and informatics. The re-formation of structure of education as well as curricula is extremely difficult, because the consequences of decisions, made at the present time will be felt just after some decades.

EXAMINATION OF ANTHOCYANIN-CONTENT OF STRAWBERRY PUREE TREATED BY HIGH HYDROSTATIC PRESSURE

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SUMMARY
Large amount of anthocyanins can be found in strawberry. Unfortunately, these compounds are very sensitive; easily lose their stability during processing and storage. Beside external factors, added ingredients (eg., ascorbic acid, sugar) can also affect the stability of anthocyanins. Before applying a new method (such as high hydrostatic pressure treatment) for preservation of strawberry, magnitude of the effect of internal and external factors on anthocyanin-content have to be evaluated.
Our results showed that right after high hydrostatic pressure treatments anthocyanin content of strawberry juices decreased, but not drastically. After storage, the anthocyanin content was almost halved compared to zero-day samples. Addition of ascorbic acid reduced the anthocyanin content, while higher sugar content preserved it.

1. INTRODUCTION

Strawberry fruit (*Fragaria ananassa*) has been shown to possess high in vitro antioxidant activity that has been positively correlated with the content of polyphenolic compounds and, specifically, anthocyanins, the type of polyphenols quantitatively most important in strawberry (da Silva et al., 2007). Moreover, anthocyanins are responsible for their appealing, bright red colour. The main problem is their low stability. In fact many factors affect the stability of anthocyanins, including temperature, pH, oxygen, enzymes, the presence of copigments and metallic ions, ascorbic acid, sulphur dioxide, sugars and their degradation products (Rodrigo et al., 2007). Application of minimal processing technologies can help to better preserve the valuable pigments by a natural way. High hydrostatic pressure treatment is a non-thermal physical preservation process, where liquid or solid foods are exposed to pressures from 100 MPa to 1000 MPa. In practice, holding times range from impulses lasting for only a few seconds to periods longer than 20 minutes.

Notwithstanding that several researchers have studied the effect of individual factors on anthocyanins, results are rather contradictory. De Rosso & Mercadante (2007) investigating the interrelationship between ascorbic acid and anthocyanins in acerola pulp and juice, that is an excellent source of ascorbic acid and anthocyanins at the same time, found that increased ascorbic acid content was the main exciting agent of loss in anthocyanin content. Whereas Kaack & Austed (1998) observed a protective effect of ascorbic acid. They stated that purging of the elderberry juice with N_2 and/or addition of ascorbic acid reduced the oxidative degradation rate of the two major anthocyanins, so ascorbic acid protected the anthocyanins.

Results of researches about surveying the effect of sugars are similarly inconsistent. While Dyrby and co-workers (2001) detected higher pigment loss in the presence of saccharides, Tsai and co-workers (2004) reported about the protective effect of saccharose, since the halving time of pelargonidine was lower in strawberry juice than in strawberry concentrate.

Anthocyanins proved to be rather resistant to HHP treatment. Patras and co-workers (2009) demonstrated that HHP treatment at 400, 500 and 600 MPa, respectively, at ambient temperature preserved well the anthocyanin content of strawberry purée. At the same time, however, inactivation of tissue enzymes responsible for the degradation of polyphenols (polyphenol oxidase and peroxidise enzymes) was not sufficient in every case, thus amount of anthocyanins may be indirectly decreased by HHP treatment (Zabetakis et al., 2000).

Based on these findings our aim was to study the effects of sugar addition, supplementation by ascorbic acid and HHP treatment on the preservation of anthocyanin content of strawberry purée during treatment and storage as well.

2. MATERIAL AND METHODS

We used deep-frozen strawberry fruits in the experiments. Fruits were thawed at room temperature then processed to purée in a sieving machine. Purée samples were centrifuged twice at 4 °C, at 1000 rpm for 20 min to remove fibres in order to prevent the oxygen remaining between fibres to influence the anthocyanin content. Then samples were supplemented to 10% or 20% refraction by glucose and 0,3% ascorbic acid by weight, respectively. Samples were put to plastic pouches, approx. 20 ml purée in each pouch, and the foil pouches were sealed. Strawberry purées packed in the pouches were pressurized in a RESATO FPU 100-2000 high hydrostatic pressure equipment at 400 and 600 MPa, respectively, for 5 or 20 min at 4 °C. A part of the samples were examined directly after the treatment, the rest after 21 days of storage at room temperature. Anthocyanin content was determined by absorbance measurement following an extraction with hydrochloric acid.

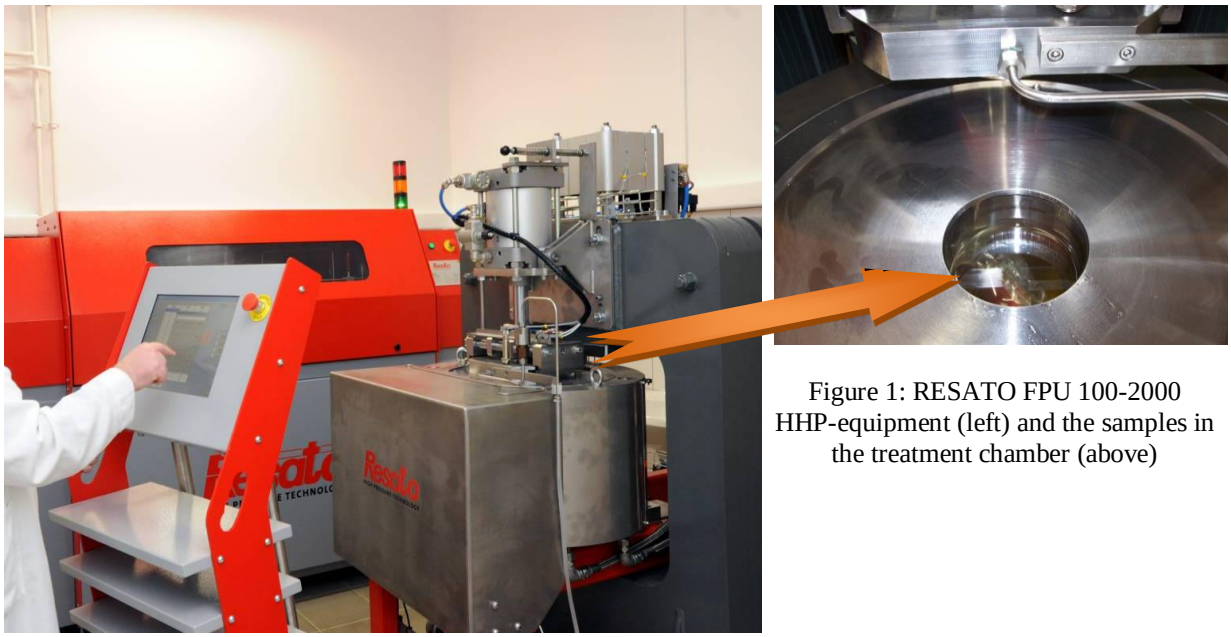

Figure 1: RESATO FPU 100-2000 HHP-equipment (left) and the samples in the treatment chamber (above)

3. RESULTS AND DISCUSSION

Figures 2. and 3. show the remaining anthocyanin content expressed in percentage based on the anthocyanin content of the initial control sample. Figure 2. presents zero-day results ($A_{treated\_0day}/A_{untreated\_0day}$), Figure 3. shows results obtained on the 21st day ($A_{treated\_21day}/A_{untreated\_0day}$). After HHP treatments at different pressure levels (400 and 600 MPa, resp.) it became obvious (Figure 1.) that the magnitude of pressure had no influence on the decrease in the anthocyanin content of juices. This was true for the length of holding times (5 min or 20 min) as well. Longer holding time caused similar changes in the decrease of anthocyanin content of strawberry juices to the ones caused by the shorter holding time. Sugar supplementation (to 10% and 20% refraction) had no significant effect on the changes in anthocyanin content either.

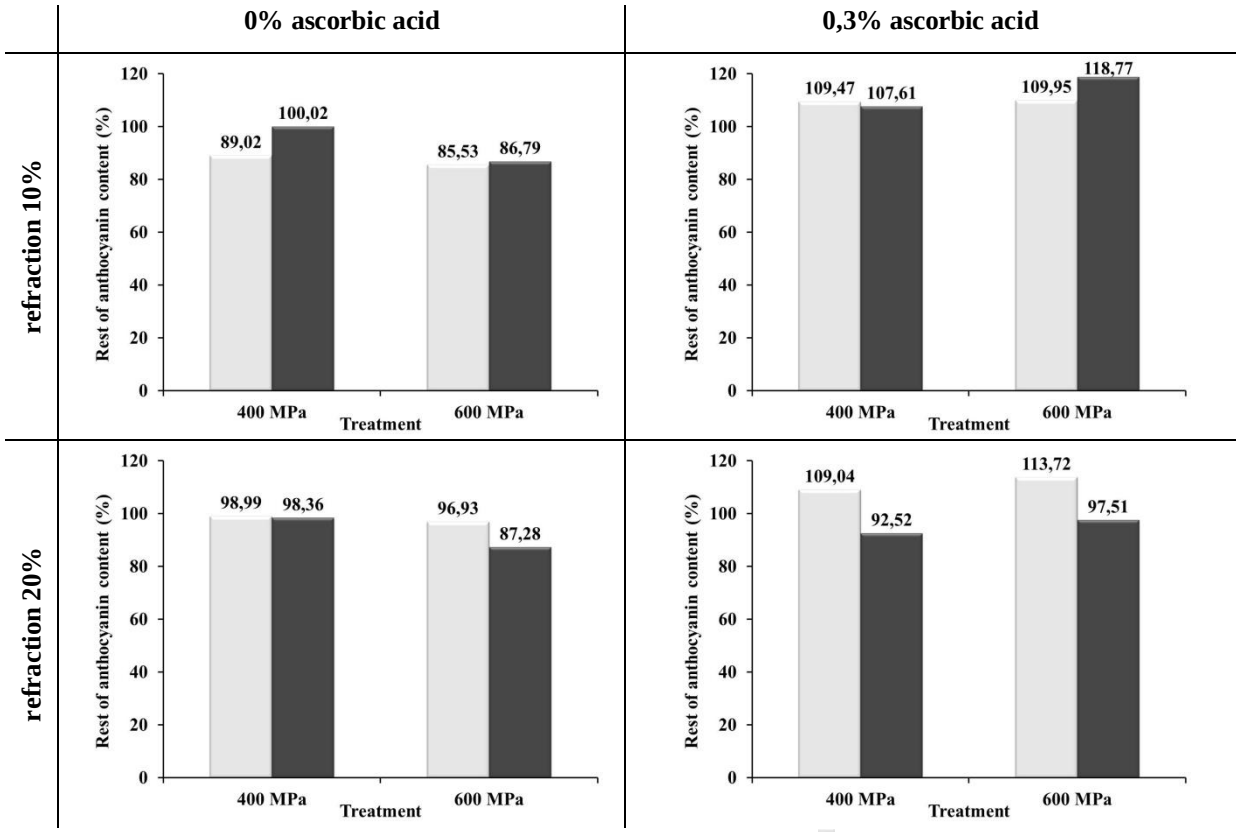

Figure 2: Effect of pressure (400 and 600 MPa) and holding time (5min: □ and 20 min: ■) on the anthocyanin content of strawberry purée samples in case of different sugar and ascorbic acid content, at 0 day ($A_{treated\_0day}/A_{untreated\_0day}$)

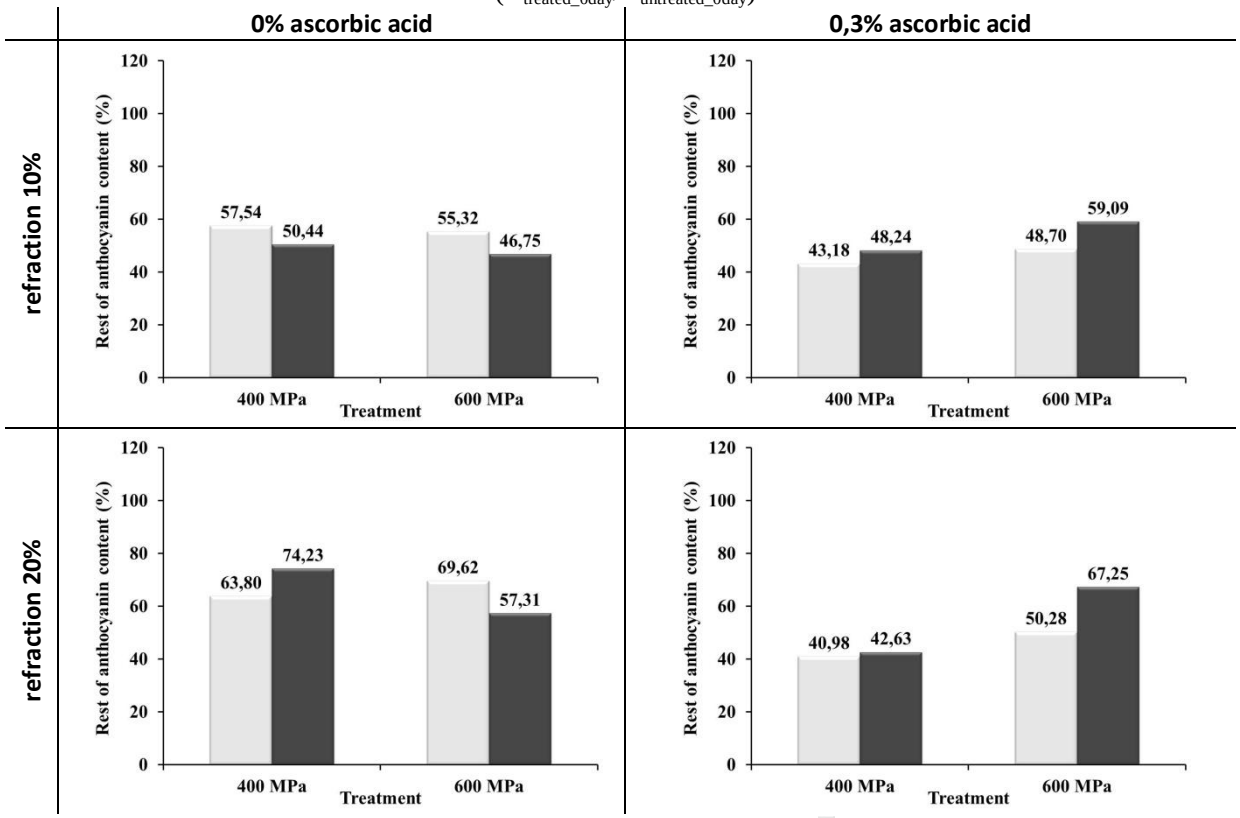

Figure 3: Effect of pressure (400 and 600 MPa) and holding time (5min: □ and 20 min: ■) on the anthocyanin content of strawberry purée samples in case of different sugar and ascorbic acid content, at 21 day ($A_{treated\_21day}/A_{untreated\_0day}$)

Table 1: Significance level of factors on quantity of anthocyanin-content of strawberry purée, analyzed by multi-way variance-analyses, right after the treatments (0 day) and after 21-day storage at room temperature

Factors	Sig. at 0 day samples	Sig. at 21 day samples
Ascorbic acid	**0.004**	0.069
Refraction	0.700	**0.037**
Pressure level	0.797	0.254
Holding time	0.479	0.415

Addition of ascorbic acid (0,3%) had more significant effect on anthocyanin content than the above mentioned treatments. Samples with higher ascorbic acid content had higher amounts of anthocyanin in every case after the treatments.

Multiway variance analysis confirmed (Table 1.) that sugar addition didn't influence significantly (p=0,700) the anhocyanin content but added ascorbic acid did have a significant effect on it (p=0,004). No systematic connection could be shown either between the effect of different pressure levels or the holding times (p=0,797 and p=0,479).

After 21 days of storage at 20 °C, anthocyanin content of samples decreased remarkably (Figure 3.). While treatments caused approximately 10% change at 0-day, after 21 days this change exceeded even 50% in some cases. Anthocyanin content of samples with added ascorbic acid decreased more compared to samples without ascorbic acid addition. This tendency was just reverse to the one observed for 0-day data concerning the effect of ascorbic acid.

Performing the multiway variance analysis with the data of stored samples as well, Table 1. shows that while pressure level (p=0,254) and holding time (p=0,415) had no significant effect on the decrease in anthocyanin content, sugar supplementation proved to be significant (p=0,037) in the change of anthocyanin content and the addition of ascorbic acid was close to being significant (p=0,069).

4. CONCLUSIONS

Our results show that the magnitude of pressure and holding time didn't affect significantly the amount of anthocyanins in the samples. This phenomenon is advantageous since initial cell count can be decreased more efficiently at higher pressures and for longer holding times without loosing the valuable anthocyanin compounds.

ACKNOWLEDGEMENT: *This research has been supported by the TÁMOP 4.2.1/B/09/1/KMR/-2010-0005 program of the National Development Agency.*

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FRANKFURTER MADE WITH REDUCED SALT CONTENT USING MICROBIAL TRANSGLUTAMINASE

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SUMMARY
Several scientific paper suggests that microbial transglutaminase is capable to reduce the salt content of brined and/or heat-treated meat products (ham, frankfurters, meat ball). In Hungary these scientific results are not known and there is no experience regarding to this application. According to this lack of knowledge, our research target was to lower the brine salt to a minimal level in popular frankfurter with the help of suitable enzyme dosage. The results according to technofunctional properties indicate that the use of 0,5% enzyme preparation can reduce the average 1.8% salt content to 1.2%.

1. INTRODUCTION

Nowadays consumers worldwide are taught to decrease their salt intake as it may help to prevent high blood pressure and coronary heart disease. There is already a Hungarian meat factory in Gyula, who launched low-fat and salt frankfurter in April (Gyulahús Ltd.) following this recommendation, but there could be much more, if there would be more research in this field showing effective ways to reach this target. As we discovered from international literature, microbial transglutaminase (E.C.: 2.3.2.13., shortly: mTG) can be a promising solution as a structure modifier, due to it's action of producing G-L bonds between food proteins (Yokoyama et al., 2004). Lee and Chin reviewed the application properties of mTG in 2010 and stated that the enzyme can improve the gelling properties of meat proteins at a low-salt level, if it is combined with functional ingridients such as sodium alginate. In this way mTG can give nearly the same textural and sensorial properties to healthier low-fat/salt meat products as regularly processed meat has. We were mainly interested to discover the possibilities to make low-salt frankfurters with mTG, because of their popularity in Hungary. Colmenero and her coworkers concluded that when mTG is used with caseinate, KCl, fibre it enhances the physicochemical characteristics of low-salt frankfurters to a level matching with normal ones. As there were no scientific results showing the effect of mTG itself on frankfurters, we focused on describing their technofunctional properties changing with enzyme dosage and salt content to give a know-how for health conscientious meat producers.

2. MATERIALS AND METHODES

Pork meat batter (1 kg) was prepared as following: half portion (235 g) of minced pork meat (80% purity) was grinded first with half portion (180 g) of ice cubs, than additives and seasonings were added in four 1-minute-long steps (1.step: 4% Na-soluprate (tetrasodium pyrophosphate, Solvent Inc., Hungary), 2. step: 1,2-1,8% salt (nitrite pickling salt containing 0.4-0.5% $NaNO_2$, Salinen Austria AG., Austria) 3. step: remaining half portion of minced pork meat, 4. step: 1.25% ground dried paprika (Kalocsai Fűszerpaprika Ltd., Hungary), 1.2% ground white pepper (importer: R-Coop 3 Inc., made in Indonesia), 160 g of pork back fat (5 mm particle size), 5. step: 0.3-1% TG H-N-F (nominal activity: 42 U/g protein, Ajinomoto GmbH.). The meat batters were partly analysed (for surface colour, extrusion, adhesiveness) partly stuffed into 21 mm diameter cellulose casings (Kalle Hungária Ltd., Hungary) and hand-linked. **Frankfurters** were pre-dried (60°C, 15min), cooked (until 72°C core temperature) and cooled in shower (until 10°C core temperature) in CS350 EL type smoking machine (Korax Ltd., Hungary). After drainage frankfurters were cooled to 5°C and stored in vacuum-package at same temperature for further investigation.

Surface colour Cielab values (lightness, L*; redness, a* and yellowness, b*) of pork meat batters and frankfurters were evaluated on a Minolta CHROMA METER CHR-400 tristimulus colour measuring system (Konica Minolta Sensing Inc., Japan). Ten replicates of the analysis were performed for each formulation.

Extrusion and adhesiveness was measured with the conical measuring head of TA. XTPlus (Stable Micro Systems, Great Britain). The cross-head pushed the 90° cone probe of spreadability rig with 2mm/sec speed into the sampling holder. Three replicates of each samples were evaluated using the official software of the instrument called Texture Exponent 32. TPA analysis was performed with TA. XTPlus (Stable Micro Systems, Great Britain) and the parameter for **hardness** was selected to analyse. Ten frankfurter cores (diam. = 21mm, height = 12mm) were axially compressed to 70% of their original height. Force–time deformation curves were derived from a 500N load cell applied at a crosshead speed of 2mm/s.

After storage overnight the samples were **evaluated with a trained panel** (9-10 members) focusing on flavour, taste and texture. The attributes were ranked on a 0-100 score scale compared to control sample (see on Fig. 4., 8.), which was fixed at the exact middle of the scale.

3. RESULTS AND DISCUSSION

3.1. Selecting the most suitable enzyme dosage for frankfurters made with 1.8% salt

The aim of the research was to follow the effect of mTG in frankfurters made with usual salt addition.

CIELab values (L*; a*; b*) were not affected by the increasing enzyme dosage. The total colour difference (ΔE*) was clearly visible (x>3.0) at 0.3% mTG level, recognisable (1.5<x<3.0) at 0.5% mTG level by meat batters and frankfurters as well.

The addition of mTG did not make significant change in the **extrusion force** of meat batter samples (see on Fig. 1.). Intrestingly the **adhesiveness** of meat batters decreased significantly with the addition of mTG even at a very low level (0.3 w/w%) as shown on Fig. 2.

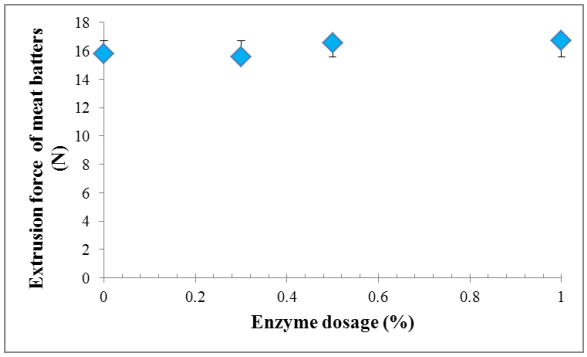

Figure 1 Effect of mTG in extrusion force by meat batter samples

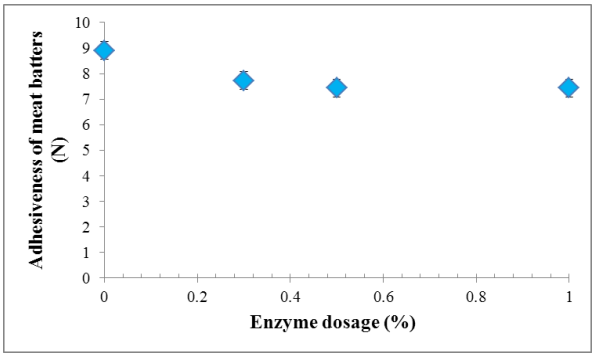

Figure 2. Effect of mTG in in adhesiveness by meat batter samples

Hardness is very important **by frankfurters** as it is in close relation with crispiness (see on Fig. 4.), one of the key features what consumers are really looking for. As shown on Fig. 3. enzyme addition led to visible change in hardness in frankfurters. The best result were reached at 0.5% enzyme dosage. **Sensorial analysis** showed that mTG enhances frankfurters in all of the measured attributes.

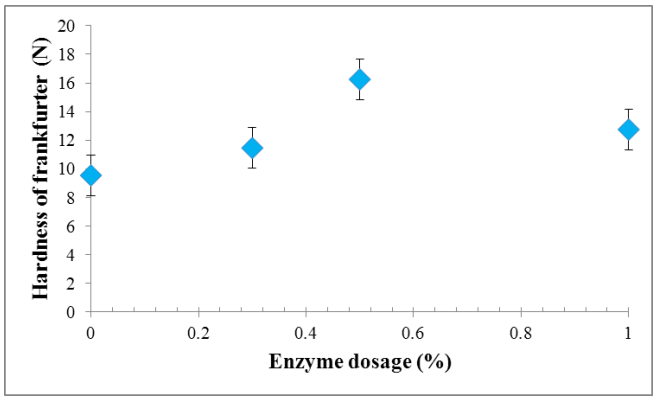

Figure 3. Effect of mTG in hardness of frankfurter samples

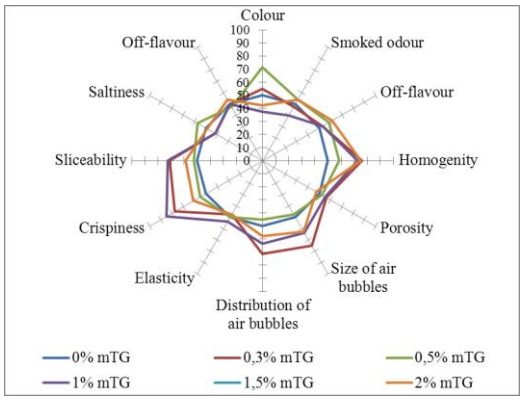

Figure 4. Changes of sensorial properties with different enzyme dosage

Our results show that 0.5% mTG is promising for making frankfurters with conventional salt addition (1.8%), but the effect of using less salt should be also investigated to know the needed and sufficient amount to produce healthier low-salt products.

3.2. Effect of salt content on frankfurters made with 0.5% mTG

CIELab values (L*; a*; b*) were not affected by the increasing salt content. The total colour difference (ΔE*) was just slightly recognisable (0.5<x<1.5) at 1.6% salt by meat batter, but clearly visible by frankfurters made with 1.4-1.8% salt addition.

Extrusion force was decreasing with increasing salt dosage, which means that in our case a meat batter with 1.2% salt was 150% more spreadable as the normally salted (1.8%) one see on Fig. 5. There was an exponential trend among salt level and extrusion grade too, as R^2=0.8998. **Adhesiveness** is following the same trend as we found exponential correlation between adhesiveness and salt level (R^2=0.9534) as shown on Fig. 6.

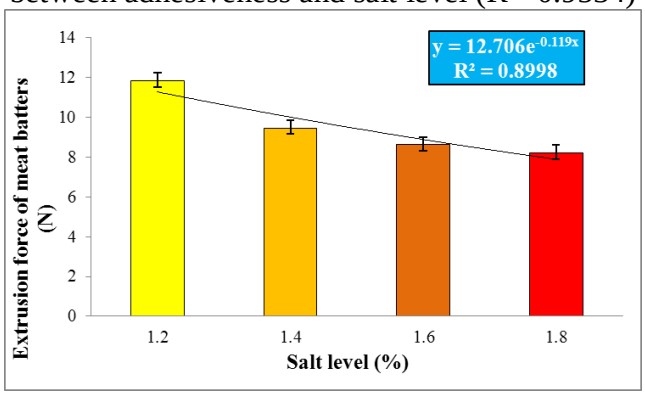

Figure 5. Effect of salt in extrusion force of meat batter samples

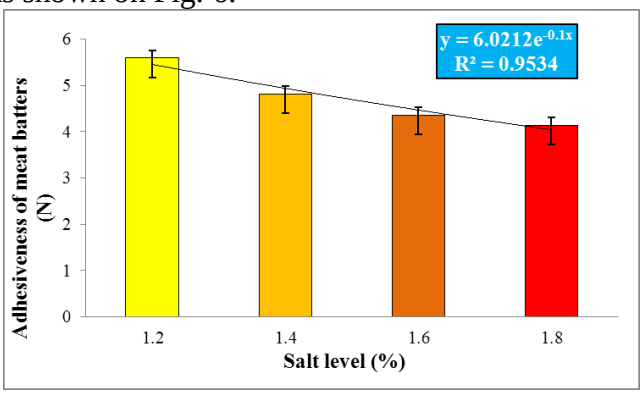

Figure 6. Effect of salt in adhesiveness by meat batter samples

Although **hardness was dependent of salt addition by frankfurters** (R^2=0.9478) as shown on Fig. 7., but it changed within a short interval (14.45-16.25N). **Sensory evaluation** (shown on Fig. 8.) revealed that frankfurter made with 1.2% salt is the most favourable for panellists in terms of saltiness, crispiness and sliceability, hence it had nearly same high scores for elasticity and porosity as products made with 1.6% and 1.8% salt.

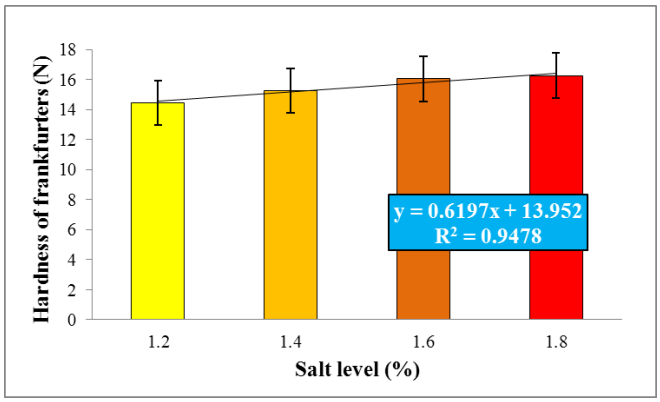

Figure 7. Effect of salt in hardness of frankfurter samples

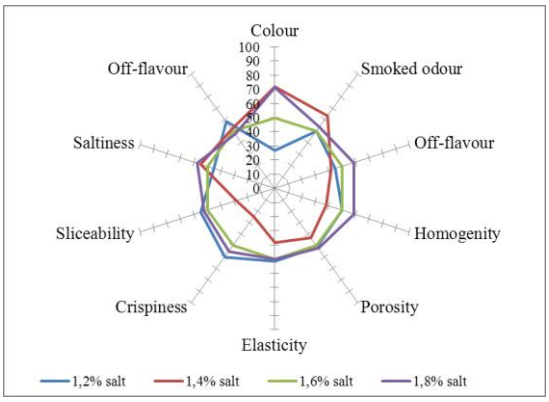

Figure 8. Changes of sensorial with different salt content

4. CONCLUSIONS

According to our research results we state that low-salt (1.2%) frankfurter with 0.5% mTG can be a good alternative to normal salted (1.8%) frankfurter without enzyme addition, as it's meat batter is easier to stuff (25% less extrusion force needed, which means more springiness and 40% less adhesiveness, which means less sticking) and the final product has better textural properties (50% more hardness) and better sensorial characteristics (20% more crispiness, 10% more sliceability) as well.

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INVESTIGATION OF THE RIPENING PROCESS OF BANANA WITH NON-DESTRUCTIVE METHODS

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SUMMARY

Maturity of bananas were characterized by using color scales during the receipt of goods so far. In this work the ripening process of ethylene-treated bananas were investigated by traditional and non-destructive methods. The firmness, starch content, chlorophyll fluorescence and color were measured and the DA-values were determined. Each maturity states were exactly delimited and quantified using the new methods, which allows faster and more objective the receipt of goods.

1. INTRODUCTION

Among the well-known and frequently consumed horticultural products, banana (*Musa cavendishii L.*) has a special role. In several parts of our world, banana is one of the major basic foodstuffs, but for example in Europe, it is a continuously demanded fresh fruit product throughout the whole year. Due to the long transport routes for banana, being largely extended in time and distance, preservation of the initial harvest status and the potential for artificial maturation, indicated as the internal and external product properties, became a highly important task. In addition, reception of banana according to quantity and quality is not fully objective; it is largely based upon a surface color reference chart (1-7). Banana fruits are still living after harvest and their physiological processes (respiration, transpiration, heat production, etc.) continue to function, significantly affecting product quality and shelf-life. Nowadays, in general, rapid, easy-to-use and non-destructive quality determination methods are needed and more and more frequently used, if available.

Several scientific approaches were done for the determination of quality changes in banana (e.g. Chen and Ramaswamy, 2001; Hashim et al., 2012; Rajkumar et al., 2012; Salvador et al., 2007; Zude, 2003), but in the everyday practice the quality determination is still based upon the product's physical parameters and the conventional color reference chart.

The main goals of this experiment were the determination of the banana ripening process (maturity phase identification according to transport, trade and commercial handling, shelf-life and overripe stage) from the green and unripe stage to the overripe stage with a special interest in the use of non-destructive quality determination methods, and additionally, the possible substitution of the well-known surface color reference chart for an objective instrumental quality determination method.

2. MATERIAL AND METHODS

'Cavendish' banana were harvested in Ecuador, bought in the Wholesale Market of Budapest at green mature state after 1 day ethylene treatment at 14 °C. Twenty bananas were kept to non-destructive measurements at 20 °C and every day (for 10 d), always at the same point, signed by felt pen, the following characteristics were measured: skin color (with Minolta CR-200 and Banana color reference chart 1-7), maturity stage by DA-index (with DA-meter), and chlorophyll activity (Walz Moni-PAM). For the traditional destructive methods 15-15 bananas were measured every day by Cone-penetrometer and Magness-taylor pressure tester.

3. RESULTS

Table 1. shows a short summary of the measured and determined results adjusted to the traditional maturity stage of bananas.

Table 1. Maturity stage of bananas and the objective characterization of their quality.

	Unripe	Ready for market	Ready for sale and for eating	Fully Ripened with aroma
Skin color	Natural green	Green and yellow color together	Fully yellow with some green at ends	Yellow with brown spots
Color scale (1-7)	1-2	3-4	5-6	7
DA-index (0-5)	1,895 - 1,45	1,44 - 0,45	0,44- 0,13	lower than 0,12
Chlorophyll F_0	1000 – 501	500 – 201	200 – 101	lower than 100
Chlorophyll F_m	4000 – 1801	1800 – 401	400 – 101	lower than 100
Chlorophyll F_v/F_m	0,678- 0,65	0,64- 0,43	lower than 0,43	
Minolta L*	61-67,5	67,6-70,5	more than 70,6	67-68,9
Minolta a*	from -15,5 to -21	from -5,5 to -15,4	from -5,4 to -1	1-2
Minolta b*	35-43,5	43,6-48	46,5-48,1	42-46,5
Cone-penetration (mm)	48 - 92	93 - 110	111 - 122	more than 123
Firmness (kg/cm^2)	3 - 0,85	0,84 - 0,57	0,56- 0,45	less than 0,44

The maturity stage has able to determine with instrumental measurements using the DA-index, the F_0 and F_m values of chlorophyll activity and the a* value of color, because the changes of these values during the ripening process are described by linear or logistic functions, and the measuring methods are fast, objective and non-destructive.

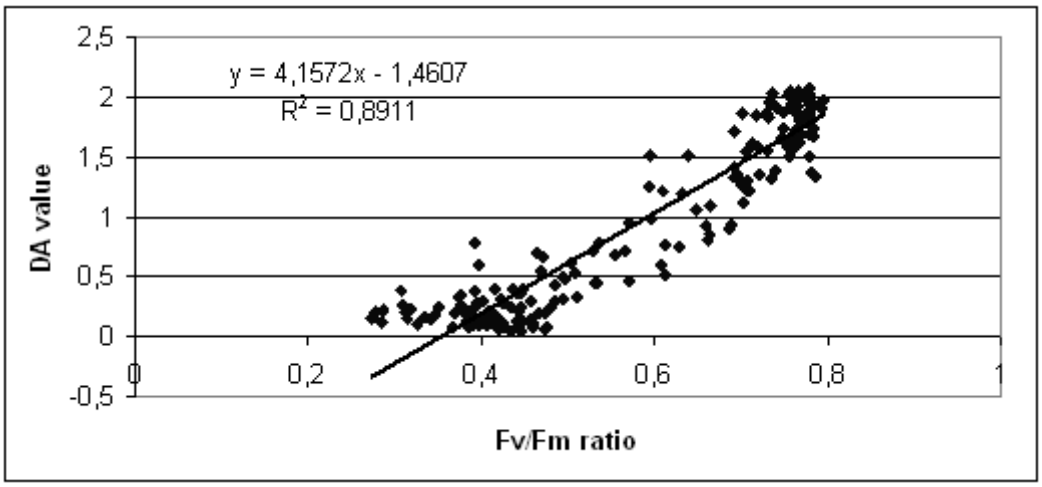

Figure 1. Correlation between DA-value and F_v/F_m ratio

Investigating the DA- index and the F_v/F_m ratio of chlorophyll activity, we find that there is a strong correlation between these values (Figure 1.). The F_v/F_m value can change between 0 and 1 while the DA index can change from 0 to 5, so the DA-meter looks like a hand-held fluorometer using approximately 4-5 times higher values than F_v/F_m. The L* and b* values of the color measurements are changing exponentially during maturation, so it's not acceptable to describe the whole ripening process just by the first segment till only the 5-6th value of the surface color reference chart. We can get also good classification using the destructive methods, but remember, these are quite slower and not repeatable techniques.

4. CONCLUSIONS

Nowadays, using non-destructive methods for classification is state of art in the postharvest sector, particularly during the reception of goods. To ensure invariant quality on the market we have to use objective measurement methods. To classify bananas during their ripening we can suggest to use either a DA-meter or a colorimeter.

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ANALYSIS OF PROPERTIES OF DRYING APPLE CHIPS

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SUMMARY

In modern society, the excellent organoleptic properties and nutritional value are important requirements of dried fruits. Our research activities include studies of the basic heat and mass transfer processes, finding relationship between physical and chemical parameters and the drying properties and determining general diffusion equations. As a first step drying properties of apple chips was determined using different drying parameters (temperature, air velocity). Based on these measurements modelling of moisture transport processes, assessment of the impact on quality of product during drying operations will be determined. Experiments were planned for the preservation of modern process technologies (such as edible packaging) application.

1. INTRODUCTION

The dried fruit-based products play an important role in our health. Implementing the changes of physical parameters used in the drying affect the energy consumption and the organoleptic, physical, chemical and microbiological properties. Our main goal is that after drying the product to comply with the required and expected by our customers, as well as lower power consumption happens during the drying process, it is particularly important to study food drying processes.

2. MATERIALS AND METHODS

2.1. Nutritional significance and the results

Standard nutritional characteristics of apple: 90% water, 9-14% carbohydrate, 0.4-1 g/100g of malic acid, and 5 mg/100g of vitamin C (URL1). The apple is the provitamin of vitamin A and beta-carotene can also be found (KÁLLAY, 2010). The apple core is contains vitamin B17, which have an inhibitory effect on the functioning of cancerous cells due to triggering the release of substances (URL2). Significant is the fruit D and vitamin F as well. A wide range of minerals found in apples including potassium, zinc, manganese, fluoride, etc.. At least 20 different ester can be detected. Apples are the most abundant source of antioxidants.

2.2. Place, time and circumstances of the tests

During the measurements, 0.01 g Precisa XB-4200CN FR analytical balance, experimental convective dryer duct, dryer cabinet and the dryer duct to control, record, and thereafter evaluate the data LabView and Microsoft Excel software. The ambient air blower on the front of the dryer duct sucks air through the heating elements and allows it to flow through the material to be dried and subsequently to the environment. The air velocity fan or hot wire anemometer measurements of the environmental parameters of the air and humidity measurement is recorded by thermohigrometer manually. The air is heated by electric heating elements provide. The drying agent - in this case apple slices – are placed on a precision balance, and hence the reduction in moisture (drying) weighing keep track of during the drying process and subsequently it is possible to determine the material drying curve. The measurements are saved in txt file using the program LabWiew. The iteration frequency can be varied and is dependent upon the duration of the drying process. The experiments are

repeated for each kind of apple (Idared, Jonagold, Golden Delicious), at a given temperature - between 50°C and 60 °C - in triplicate, with an air speed 1 m/s was performed. The first step is to adjust the proper test parameters (velocity and temperature) on the LabView program, the program was initiated without a sample to be tested. At the time the apples were placed in the balance of the dryer duct, the air speed and temperature are at the pre-determined parameters.

3. RESULTS AND DISCUSSION

After the drying of apple slices with the data recorded by the LabView program was evaluated using Microsoft Excel and determined drying curves for each process. The drying rate curves in this case, regarding moisture and time were calculated.
Determination of the moisture ratio according to the following equation (1):

$$MC = \frac{MC - MCe}{MCo - MC_e} \quad (1)$$

where
MC is the actual moisture content
MC_o is the initial moisture content
MC_e is the equilibrium moisture content (Hui et al., 2008.)

3.1. Comparison of different varieties of apple drying curves

The Figure 1 shows the curves of the apple varieties drying at 50 °C, and Figure 2 shows how the curves were at 60 °C.

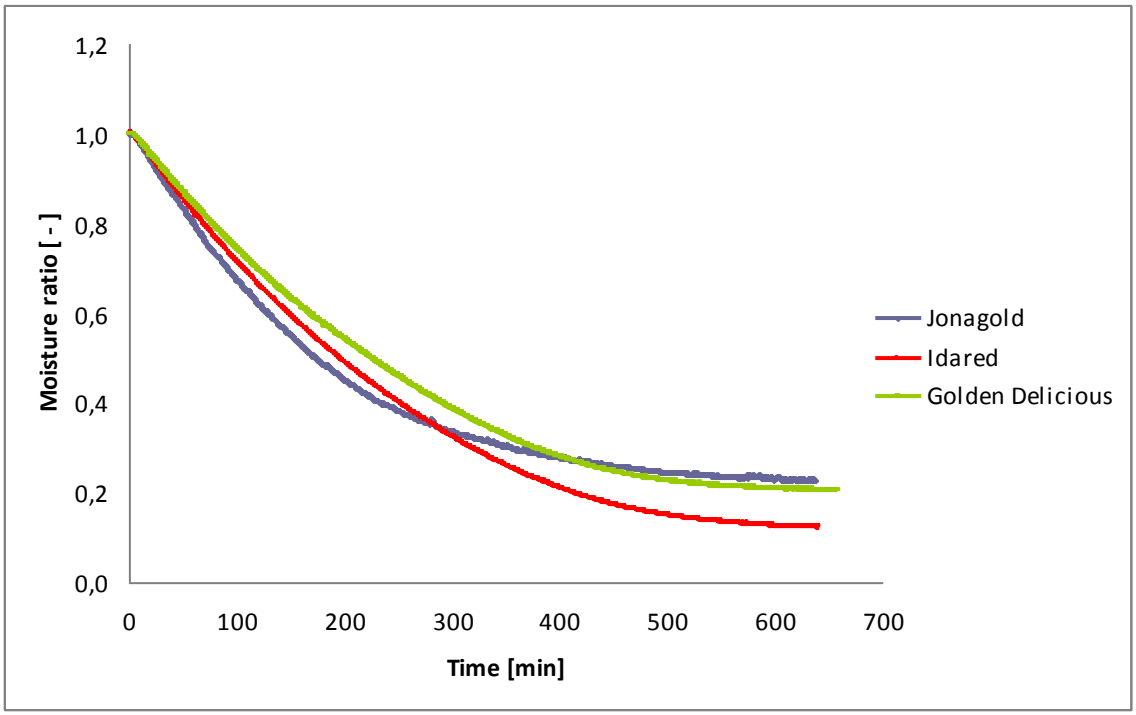

Figure 1: Drying curves for the different varieties of apple at 50 °C.

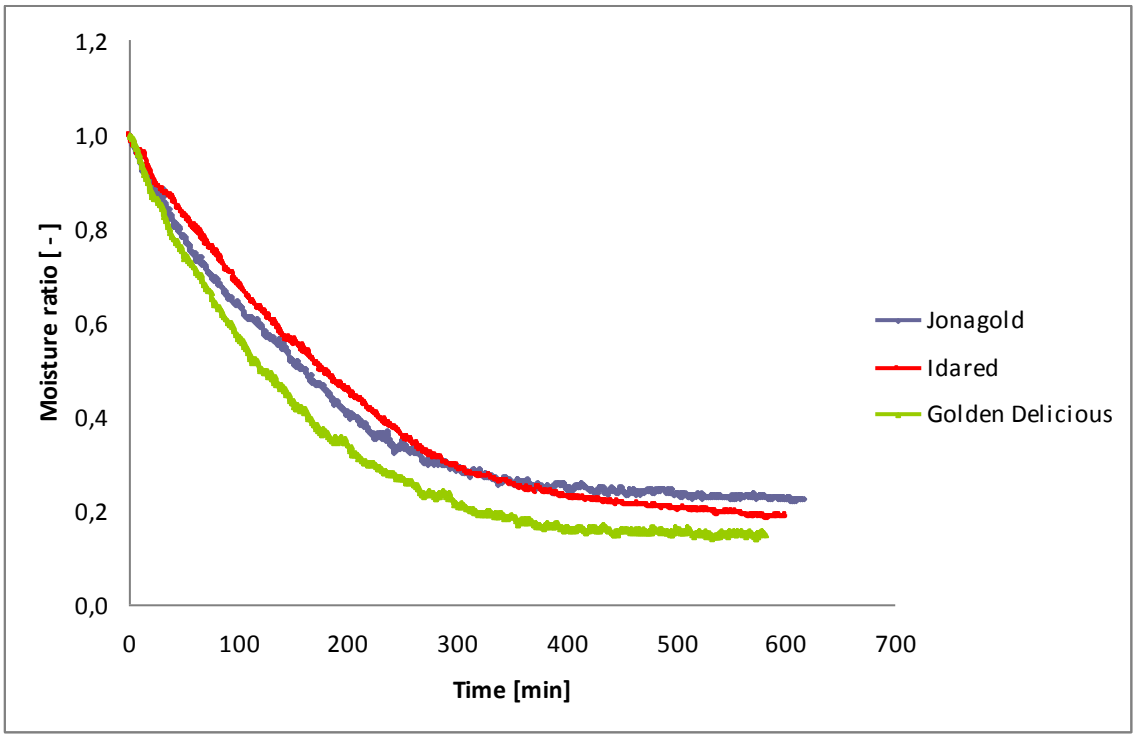

Figure 2: Drying curves for the different varieties of apple at 60 °C.

First, it has to be clear that the different varieties in the comparison have varying drying curves and mass action permanence in relation to time vary widely at the two different control temperatures. For the Idared sample at 50 °C for 620 minutes the constant weight is observed. In comparison to the Jonagold sample had a constant weight 15 minutes earlier, the Golden Delicious sample was reached 20 minutes later. A significant difference can be seen, which shows the results at 60 °C for drying. A temperature increase of interesting twists and turns occurred at 50 °C, the Golden Delicious samples proved to be most effective at this temperature for 510 minutes, or eight and a half hours of drying. Compared to the Golden Delicious Idared samples reached a constant weight of one hour later, the Jonagold samples were one and a half hours later.

4. CONCLUSIONS AND RECOMMENDATIONS

Based on studies done in finding that the 50 °C to 1 m / s air velocity there is a possibility of the sample rehydrating after drying. So we can say that in addition to these parameters dried product does not meet consumer expectations, due to an unstable product (there is a risk of mould growth during storage, etc). Therefore, - where the air velocity does not aim to increase - at least 60 ° C, it is appropriate to set the temperature of the drying air.
The measurement results also show that if the air velocity remains drier 1 m/s, but the temperature of 60 °C was increased from 50 °C, then a greater energy input for drying as required, but the drying time is shortened.

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URL[2]: http://www.vitaminsziget.com/cikk.php?id=779

RSM AND ANN MODELING OF MASS TRANSFER DURING OSMOTIC DEHYDRATION OF SUGAR BEET

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SUMMARY

Osmotic dehydration is widely used for the partial removal of water from plant tissues by immersion in a hypertonic (osmotic) solution. During osmotic dehydration, water removal from the plant tissue is always accompanied by the simultaneous counter flow of solutes from the osmotic solution into the tissue. In this study, a comparison between artificial neural network (ANN) and response surface methodology (RSM) was done in the prediction of the mass transfer parameters during osmotic dehydration of sugar beet. Mean square error and coefficient of determination were used as the measure of predictive capabilities of the two methodologies compared. The results showed that properly trained ANN model is found to be more accurate in prediction as compared to RSM model.

1. INTRODUCTION

Osmotic dehydration is a method of partial removal of water by soaking foods, mostly fruits and vegetables, in hypertonic solutions. The driving force for the diffusion of water from the plant tissue into the solution is difference between osmotic pressures of the hypertonic solution and plant tissue.

The diffusion of water goes together with simultaneous counter diffusion of solutes from the solution into the plant tissue (Lazarides et al., 1995). Leakage of the natural solutes from the plant tissue occurs because the cell membranes of plant tissue responsible for osmotic transport, is not perfectly selective but this flow is negligible, even though it may be imperative for the sensory and nutritional properties of the product (Fito., 1994; Tsamo et al., 2005).

Solute selection and concentration depend on numerous factors but the most important are solute solubility, its effect on sensory quality properties as well as its cost (Yao Z et al., 1996). Sugars and salts are two most widely used solute types for osmotic dehydration, with relevance for sucrose and sodium chloride (Lenart and Flink, 1984; Ponting, 1973). As it was reported earlier, combined solutions of these two substances can be used to enhance water removal with low solids gain by the products (Sachetti et al., 2001).

Sugar beet (*Beta vulgaris saccharifera* L.) is good source of dietary fibers that are necessary in human nutrition and it can be used as complement for bran (for example in breakfast cereals). Additionally, the whole sugar beet can be used as a substitute for sugar. Diets rich in fiber are initiate a wide variety of health benefits.

Response surface methodology (RSM) is an empirical statistical modeling procedure in employment for multiple regression analysis using data obtained from planned experiments. Artificial neural network (ANN) can be used as an alternative to the polynomial regression based modeling tool, which offers the modeling of complex nonlinear relationships. The ANN model is potentially more accurate by including all the experimental data.

In this study, a response surface methodology and artificial network model models were developed to predict the mass transfer (water loss and solid gain) during osmotic dehydration of sugar beet.

2. MATERIALS AND METHODS

2.1. Materials

Sugar beets were obtained from ‘‘Bačka’’ sugar factory (Vrbas, Serbia) and stored at 4°C prior to experiments. Sugar beets were thoroughly washed with water to remove soil and other debris. After peeling it was cut into long thin strips. The cossettes thus obtained are generally about 3–4 mm thick and 4–5 cm long. The average moisture content of the sugar beet was found to be 75% on a wet basis. Sucrose and sodium chloride were food grade commercial products and purchased from local supermarket.

2.2. Osmotic dehydration

The osmotic dehydration was carried out in shaker fitted inside a thermostatically controlled chamber. 250 ml beakers, filled with osmotic solution, were used as dehydration vessels, in which samples (about 20 g) were placed. Sugar beet cossettes were totally submerged in solution during the osmotic treatment. In order to avoid dilution of osmotic solution and subsequent decrease of driving force for osmotic dehydration, the weight ratio between sample and osmotic solution was 1:10 (Saputra, 2001). In each of the experiments fresh osmotic solutions were used. At each sampling time cossettes were taken out and washed with fresh running water to remove excess solution. Washed samples were drained and gently blotted with adsorbent paper and weighed. Moisture and dry matter content of the samples were determined by drying at 70 °C for 24 h in an oven. All experiments were done in triplicate and the average value was taken for further calculations.

3. RESULTS

A second-order central composite design (CCD) with four factors (immersion time, sucrose and sodium chloride concentrations, and temperature) at five levels each to take into account the individual and interaction effects of the factors. The experimental design included 30 experiments (divided into three blocks) with five replications of the center point (Morgan, 1991). The factor variables and their range are: immersion time (30–240 min); sucrose concentration (30–70%, w/w); sodium chloride concentration (0–8%, w/w) and temperature (30–50 °C). Water loss (WL) was expressed as the net loss of water from the fresh cossettes after osmotic treatment based on initial sample weight and solids gain (SG) was calculated as net uptake of solids by osmosed sample based on initial sample weight (Jokić et al., 2006; Le Marguer, 1988).

The second-order polynomial equation was fitted with the experimental results obtained on the basis of CCD experimental design. The results of the RSM indicate that the equation adequately represented the actual relationship between the independent variables and the examined responses, i.e. water loss and solid gain. Regression equation coefficients for water loss and solid gain are 0.97 and 0.96, respectively. On the other hand mean square error is in range between 0.2 for water loss and 3.0 for solid gain. The higher value obtained for solids gain, because small differences between experimental and predicted values after they are divided by low predicted values of SG cause a great increase in mean square error.

ANN-based process model was developed using the feed-forward network architecture namely, multilayer perceptron (MLP) with sigmoidal function. As learning rule Levenberg-Marquardt algorithm is selected. Four inputs for the network are factor variables, i.e. immersion time; sucrose concentration; sodium chloride concentration and temperature, whereas output values are water loss and solid gain. To improve the behavior of the ANN,

input and output data were normalized. Two independent network architectures are tested one for WL and the other for the SG.

The optimum number of hidden nodes was chosen upon minimizing the difference between predicted ANN values and desired outputs, using coefficient of determination and mean square error during testing of the network. In the case of the water loss optimal network architecture is 4-6-1, i.e. network with six neurons in hidden layer. Coefficient of determination is 0.999, and mean square error is 0.005. In the case of the solid gain coefficient of determination is slightly lower 0.986, while mean square error is 0.012 and the optimal network is 4-7-1, network with seven hidden neurons. To obtain higher values of coefficient of determination and lower values of mean square error, for both WL and SG, it can be noted that the optimal number of neurons in the hidden layer is as mentioned earlier and also that a greater number of hidden neurons increases the network structure complexity and does not improve the network prediction capabilities.

Prediction performance of RSM and ANN model (WL and SG) are shown in figure 1. The predicted values were very close to the desired values in most cases, although SG prediction was not as good as that for WL. Compared to RSM model the ANN model predictions are distributed nearby to the line of perfect prediction. Thus it is obvious conclusion that the ANN model shows a significantly higher generalization capacity than the RSM models. This higher predictive precision of the ANN can be attributed to its ability to approximate the nonlinearity of the system, whereas the RSM is restricted to a second-order polynomial. Generation of ANN model requires a large number of iterative calculations, but it in the case of the RSM model it is only a single step calculation. On the other hand ANN model may require a time-consuming computational procedure. RSM model quality is possibility to estimate the significance of each coefficient, i.e. linear, quadratic and interactions effects of selected variables by Student′s t-test and p-values. In the case of the ANN model estimation of the estimation of variables significance can be done by applying Garson or some other algorithm based on the values of weights of trained network (Jokić et al., 2011).

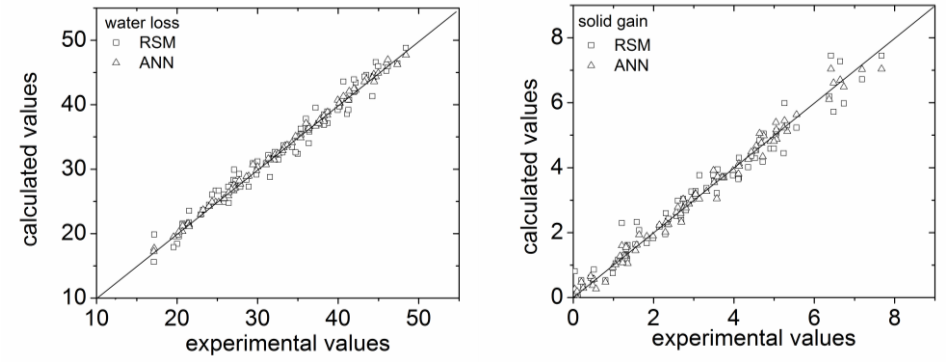

Figure 1: Water loss and solid gain model prediction performance

4. CONCLUSION

This study compares the performance of response surface and artificial neural network methodologies with their prediction capabilities using the experimental data based on the A second-order central composite design in the osmotic dehydration process of sugar beet in combined sucrose and sodium-chloride solutions. An effort has been made to model the osmotic process using response surface and artificial neural network approaches. The designed nature of the RSM is useful to reveal the factors influences from the coefficients in the regression models but ANN model has shown improved predictive capabilities although even RSM has satisfactory predictive power. As compared to multiple regression models (RSM), ANN models yield a better fit of experimental data. Further investigations are needed to evaluate sensory and physical properties of products.

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CHANGES IN FREE AMINO ACID AND BIOGENIC AMIN CONTENT OF HIGH HYDROSTATIC PRESSURE TREATED CHEESES

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SUMMARY

Amino acids and biogenic amines are important bio-molecules in nutritional point of view. Consumption of biogenic amines in higher amounts may cause food related pseudo-allergic reactions in sensitive individuals. Many methods have been developed to reduce formation of BAs in food e.g. using high hydrostatic pressure, and selected bacterial strains. The aim of this work was to study (1) the effect of high hydrostatic pressure (HHP) treatment; (2) the influence of the selected lactobacillus strain (Lactobacillus curvatus 2770) and an industrial starter culture (Choozit) on the formation of free amino acids and biogenic amines in cheese during 5 weeks of storage. Free amino acid and biogenic amine analysis was carried out by an automatic amino acid analyzer. HHP treatment (500 MPa, 10 min) and the selected lactobacillus strain were effective in reduction of biogenic amine content (L. curvatus by 20%; Choozit by 46%) in cheese by the end of storage compared to the control samples.

1. INTRODUCTION

Cheese is considered to be a good source of proteins, vitamins and minerals. In cheese, casein degradation occurs during maturation leading to the accumulation of free amino acids that can be converted into biogenic amines, so cheese represents an ideal environment for biogenic amine production. The most important biogenic amines in cheese are histamine, tyramine, putrescine and cadaverine (Stratton et al., 1991). High amounts of biogenic amines (BAs) may cause food related pseudo-allergic reactions in sensitive individuals. Several efforts have been made in food science and in the food industry to reduce formation of BAs in food. Using high hydrostatic pressure (HHP) treatment (Calzada, 2013) or selected starter culture (Zhang et al., 2013) are the most encouraging methods for the reduction of biogenic amine content in food. HHP treatment is a non-thermal preservation technology that extends shelf life while retaining the original flavour and characteristics of food (Patterson, 2005).

The aim of this work was to study the effect of HHP treatment and the influence of the selected lactobacillus strain (*Lactobacillus curvatus* 2770) and an industrial starter culture (Choozit) on the formation of free amino acids and biogenic amines during cheese ripening and storage.

2. MATERIALS AND METHODS

2.1. Cheese manufacture

Ten litres of pasteurized, non-homogenized bovine milk with 2.8% fat content (Dabastej Ltd., Dabas, Hungary) were heated to 30 °C during continuous agitation (15 traverses/min) in a laboratory-scale stainless steel jacketed cheese vat (FT20 Cheese Vat, Armfield Ltd., UK). *Lactobacillus curvatus* 2770 and a commercial Choozit Cheese Culture MA4001 (Danisco A/S, Denmark) were used to inoculate the milk. The home made cheeses were vacuum packaged in Cryovac BB4L foil bags (Sealed Air Corporation, USA) (oxygen permeability 30 $cm^3/m^2/24$ h at 23°C, 0% RH and 1 bar) and stored at 13±2°C in a cooling cabinet (J 600-2, Thermotechnika Ker. Ltd., Hungary) for 5 weeks. Samples were taken every week.

2.2. High hydrostatic pressure treatment

The cheese ripening lasted for 3 weeks (13 ^{o}C) in order to allow biogenic amine formation. At the end of the 3rd week, the halves of cheese wheels were pressurized at 500 MPa for 10 min at room temperature (max. temperature in the pressure chamber 26.8 °C) in a FPU-100-2000 type equipment (Resato International B.V., the Netherlands). HHP treated samples were stored for 2 more weeks.

2.3. Free amino acid analysis

Cheese samples (2 g) were extracted with 10 cm^3 of 10% trichloroacetic acid for 1 h at room temperature, at 100 rpm using a Laboshake (Gerhardt, Germany). Samples were filtered through a 0.25 µm membrane filter (Nalgene, USA). Free amino acid analysis was performed with an AAA 400 Amino Acid Analyser (Ingos, Czech Republic) equipped with an IONEX OSTION LCP5020 ion-exchange resin (200×3.7 mm) column, using Li^+ buffers.

2.4. Biogenic amine analysis

The same extracts were used for the determination of biogenic amines as for the free amino acids. BA analysis was performed with the same Amino Acid Analyser equipped with an Ostion LG ANB ion-exchange resin (70×3.7 mm) column, using Na^+/K^+ buffers (Simon-Sarkadi, 2012).

3. RESULTS

3.1. Free amino acid content of cheese

The free amino acid (FAA) content of control samples continuously increased during the 5 weeks of storage. The FAA content ranged between 648-2428 µg/g, and 624-1431 µg/g in samples inoculated with *Lactobacillus curvatus* 2770 and Choozit cheese culture, respectively (Fig.1). The ratio of single amino acid composition was significantly different in the two types of cheese. The main amino acids in cheese inoculated with *Lactobacillus curvatus* 2770 were Glu, Orn, Lys, Leu, and Gaba and in cheese inoculated with Choozit were Leu, Gaba, Phe, Orn, Gln, Asn, and Lys, in decreasing order. HHP reduced the free amino acid content of cheese after pressure treatment (3rd weeks) by 40% and by 29% produced with *L. curvatus 2770* and Choozit, respectively. During storage a 14% increase in FAA was measured in the case of cheese samples made with *L. curvatus* 2770 while in cheese made with Choozit FAA content decreased by 25%.

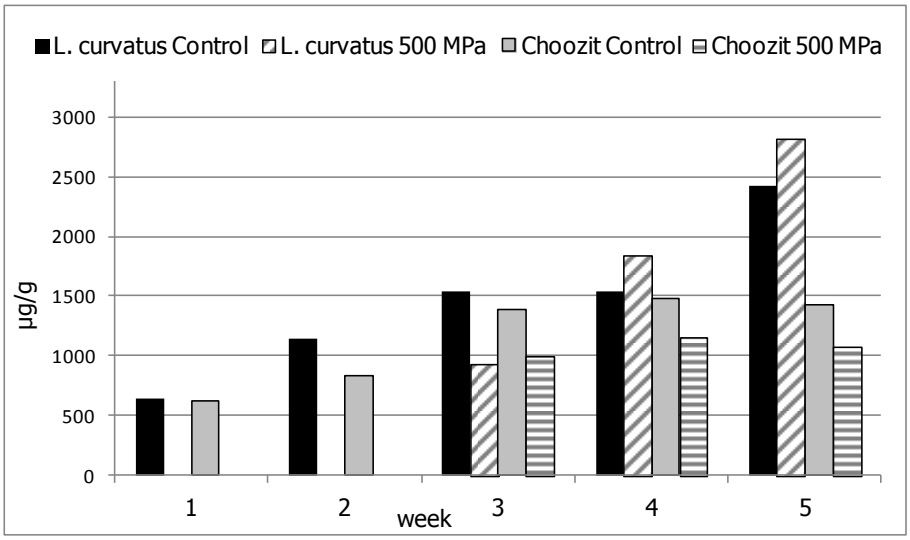

Figure 1: Changes of free amino acid content in cheese samples during five weeks of storage

3.2. Biogenic amine content of cheese

The total biogenic amine (BA) content ranged between 25-40 µg/g, and 3-140 µg/g in control samples inoculated with *L. curvatus,* and Choozit cheese culture, respectively. The total amounts of BAs were significantly lower in cheeses produced with *L. curvatus* 2770 than in cheeses made with industrial starter culture (Choozit). Cadaverine (45%) and putrescine (38%) were the major amines in cheeses with selected *L. curvatus* 2770 (Fig. 2) while tyramine (68%) was the predominant BAs in cheese manufactured with Choozit cheese culture (Fig. 3) and the latter cheese contained higher amounts of histamine (15%) and putrescine (15%). HHP treated samples contained 20% (*L. curvatus* 2770) and 46% (Choozit) less BAs compared to that of untreated samples by the end of storage. The BA composition was much better in respect to tyramine and histamine content of cheeses made with selected lactobacillus strain (*L. curvatus* 2770), than in cheeses fermented with the industrial Choozit cheese culture.

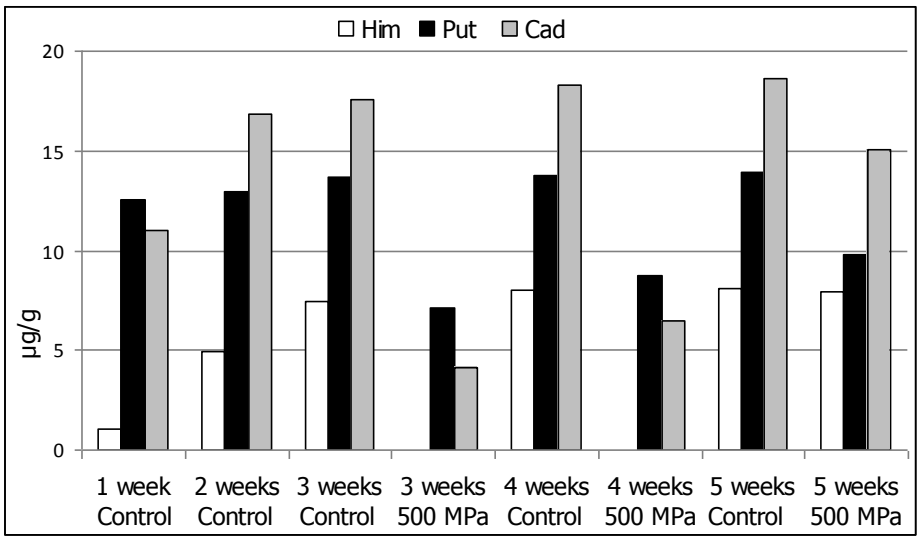

Figure 2: Changes of biogenic amines in cheese samples inoculated with *Lactobacillus curvatus* 2770

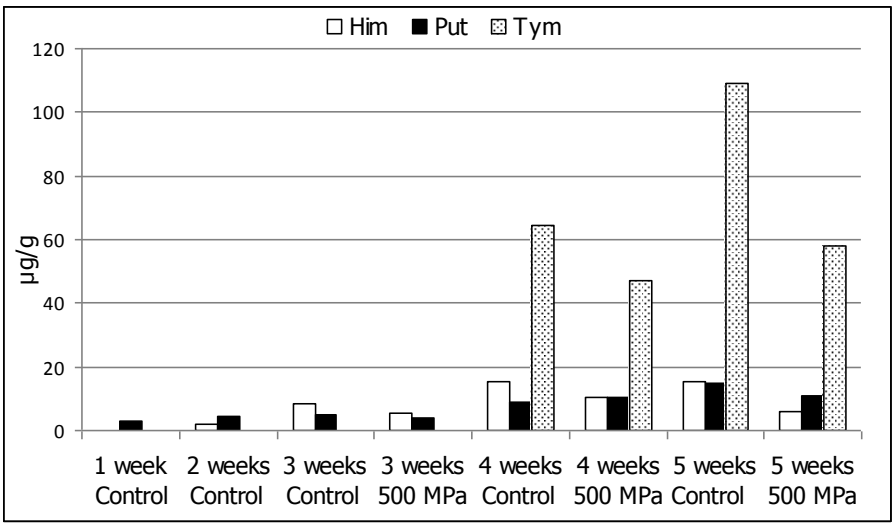

Figure 3: Changes of biogenic amines in cheese samples inoculated with Choozit

4. CONCLUSION

Our result indicates that it is recommended to use selected starter cultures to obtain a healthier product regarding the biogenic amine content and composition of cheeses. HHP treatment improved the microbial quality of the cheese and it was effective in the reduction of BA formation during storage.

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EFFECT OF HIGH PRESSURE TREATMENT ON LIQUID WHOLE EGG

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SUMMARY

In our tests, we artificially infected liquid whole egg samples with Salmonella Enteritidis, Listeria monocytogenes, and Staphylococcus aureus bacteria, and then treated the samples in "Food Lab900" high hydrostatic pressure (HHP) instrument for 3–17 min at 200–400MPa. Subsequently, the change of the viable cell count of the specific bacteria has been tested. In addition to the samples infected with various bacteria, non-infected samples were also treated in each test and the change in viable cell count, colour and viscosity of the samples upon the effect of the treatment. In summary, it can be concluded that in each test of our investigations, the viable cell count of S. Enteritidis critical for egg products is reduced significantly, while the reduction of the total viable cell count was around two magnitudes. Additionally, based on our results, microbial destruction, reduction of enthalpy (denaturation of egg white) caused by the treatment at HPP, and colour change are primarily affected by the pressure level, while the changes in rheological properties are also significantly affected by the duration of high pressure treatment ($p < 0.05$).

1. INTRODUCTION

Numerous researches have been conducted today to develop procedures replacing onventional liquid egg pasteurization technologies (heat treatment at 60–65◦C for 5–10 min). One such procedure includes treatment of liquid egg products at high hydrostatic pressure (HHP. Previous research has shown that HHP technology is suitable for destruction of numerous pathogenic micro-organisms in egg products (Farr, 1990).

The use of HHP technology allows better preservation of native characteristics of food raw material with similar antimicrobial efficacy to heat treatment and its beneficial effect has been demonstrated with many foods sensitive to heat treatment (Seregély et al., 2007). An additional advantage includes that in HHP procedure, liquid foods are treated in the packing material to avoid potential post-infection of the product. Furthermore, for pasteurization of bulk material (exceeding 1 kg) in contrast to heat treatment, no heat shock effect induced by low warming-up rate is expected in case of HHP procedure since antimicrobial effect occurs momentarily at the same time at all points of food.

For treatment of liquid whole egg in addition to the purpose of achieving the satisfactory microbiological condition, it is important that the product should preserve its beneficial organoleptic and functional properties. Deterioration of such properties such as viscosity of product is related to coagulation of specific egg proteins induced by HHP.

In this work, our purpose was to investigate the effect of treatment at HHP not significantly deteriorating the calorimetric properties (treating pressure below500MPa) on the microbiological and physical characteristics of whole liquid egg.

2. MATERIAL AND METHODS

2.1. Samples and structure of experiments

In our measurements, we tested unpasteurized homogenized liquid whole egg samples, and for each test, we artificially infected the liquid whole egg samples with *Salmonella* Enteritidis NCAIM B2052, *Listeria monocytogenes* NCAIM B1371, and *Staphylococcus aureus* NCAIM B2278 bacteria to achieve 10^8 CFU/mL viable cell count in the samples. The strains were incubated overnight at 37°C were in the stationary phase. About 50mL of each

sample was treated in "Food Lab900" HHP instrument (model S-FL-850-9-W, STANSTED Fluid Power Ltd., UK) for 3–17 min at 200–400MPa using Central Complex Rotation Design.

The main advantage of this experimental approach is that we had to perform less experiment to obtain information sufficient for statistically acceptable results since only 11 tests are needed to establish 1 model. For approximation, we used the response surface obtained based on secondary polynomial model. Experiments were conducted in random order and data were analysed by the Unscrambler v 9.1 (CAMO PROCESS AS, OSLO, Norway) software. In the general form of the secondary polynomial model used in this study, there were two X variables.

$$Y = \beta_{PP} + \beta_P P + \beta_2 T + \beta_{PP} \cdot P^2 + \beta_{TT} T^2 + \beta_{TP} \cdot P \cdot T,$$

comprises linear P, T expressions and quadratic P^2, T^2 expressions. T variable represents the treatment time and P the treatment pressure. Y is an independent variable to be modelled. β_T , β_P, β_{TT} , β_{PP}, β_{TP} expressions are the regression coefficients of the model. The samples were cooled to 4°C prior to the experiment; during the treatment, the samples were cooled and their temperature was below 15°C.

2.2. Viable cell count test

In our tests, we tested the effect of treatment time and treatment pressure on the total viable cell count and *S.* Enteritidis, *L. monocytogenes*, and *S. aureus* viable cell count within the tested range. Dilution plate pouring was performed for each testing to measure viable cell count. Tenfold serial dilution was performed from the samples using sterile water, and then microbial count of samples was measured by plate pouring with Nutrient agar (Merck) (Brain Heart (Merck) agar for L. *monocytogenes*). Plates were incubated at 37°C (30°C for total viable cell count) for 48 h and the number of colonies grown was counted by a colony counter.

2.3. Colour test

Colour analysis was performed using Minolta Chroma Meter CR-200 model tristimulus colour analyser for measuring reflected-light colour. In CIELAB system, L∗ is lightness (black point L∗ = 0, white point: L∗ = 100), a∗ is characteristic to red-green colour and b∗ is the blue-yellow colour (sign: +a∗ red, −a∗ green, +b∗ yellow, −b∗ blue).
The CIELAB colour stimulus space uses vector values to characterize chromaticity. The colour difference is the most important value of the CIELAB system.

2.4. Viscosity measurement

Tests were performed with Physica MCR 51 (Anton Paar Hungary) rotation viscosimeter, by a measurement system comprising CC 27 (cylinder with 27 mm) measuring body and ST 24-2V-2V-2D measuring head. Viscosity of sample solutions was tested with 600 s−1 deformation rate at 20°C. All samples were measured in seven parallel.

3. RESULTS AND DISCUSSION

Table 1 shows the reductions of each bacterial count and the total viable cell count as well as the rate of colour change observed in each sample compared to the control sample. Furthermore, Table 1 shows the viscosity of samples treated differently.

Since the amount and nature of samples did not allow direct analysis of the colour status of liquid egg samples, this measurement was performed through a plastic foil. We tried to minimize the resulting difference by performing the preliminary calibrations not simply with the white test piece (ceramic sheet), but we placed a clean plastic foil below the measuring head. Ten replicates were measured with the sample of each test. Extent of colour change is determined by comparison of treated samples with control (untreated) sample.

Table 1: Specific test parameters and corresponding results.

			Results, mean (±SD)					
			$\lg(N/N_0)$, (CFU/mL)			Total viable cell count (N)		
Test	P	T	*S. enteritidis*	*L. monocytogenes*	*S. aureus*	(CFU/mL)	ΔE^*ab	Viscosity (Pa s)
1	300	10	5.28 (±0.23)	1.51 (±0.12)	2.01 (±0.19)	2.37 (±0.21)	4.31 (±0.031)	0.01271 (±0.0005)
2	200	10	4.89 (±0.17)	0.90 (±0.18)	1.84 (±0.17)	2.41 (±0.15)	1.50 (±0.110)	0.01249 (±0.0005)
3	300	17	6.41 (±0.18)	1.67 (±0.19)	2.28 (±0.23)	2.21 (±0.16)	5.84 (±0.080)	0.01913 (±0.0010)
4	230	5	4.91 (±0.22)	0.94 (±0.15)	1.83 (±0.18)	2.40 (±0.20)	2.57 (±0.060)	0.01605 (±0.0007)
5	370	5	5.96 (±0.30)	1.95 (±0.17)	2.34 (±0.32)	1.99 (±0.17)	5.49 (±0.040)	0.01707 (±0.0008)
6	230	15	5.00 (±0.13)	0.97 (±0.18)	2.00 (±0.16)	2.35 (±0.21)	5.00 (±0.032)	0.01298 (±0.0005)
7	300	10	5.31 (±0.23)	1.65 (±0.12)	1.93 (±0.18)	2.32 (±0.21)	4.33 (±0.070)	0.01820 (±0.0009)
8	400	10	5.31 (±0.25)	1.98 (±0.20)	2.63 (±0.26)	1.36 (±0.12)	5.33 (±0.081)	0.01428 (±0.0006)
9	300	3	5.75 (±0.31)	1.41 (±0.13)	0.92 (±0.09)	2.34 (±0.17)	5.46 (±0.065)	0.01817 (±0.0009)
10	300	10	4.99 (±0.19)	1.59 (±0.14)	1.95 (±0.17)	2.25 (±0.19)	4.58 (±0.030)	0.01729 (±0.0009)
11	370	15	6.11 (±0.20)	1.96 (±0.19)	2.47 (±0.18)	1.65 (±0.11)	4.48 (±0.040)	0.01712 (±0.0005)

Notes: P, treatment pressure (MPa); T, treatment time (minutes); N, total viable cell count post treatment (CFU/mL); N_0, total viable cell count pre-treatment (CFU/mL).

By comparing the test result many times, significant differences can be seen in microbiological and physical changes induced by HHP treatments of various degrees and times. Effect of pressure level and time can be observed in comparison of tests in which one variable was included with the minimum and maximum value of the test range, but the other variable is the same in the two tests.

For example, comparing Test 2 (treatment pressure (P): 200MPa, treatment time (T): 10 min) and Test 8 (P: 400MPa, T: 10 min), it can be seen that changes in pressure result in more than 0.5 magnitude difference in reduction of viable cell count of S. aureus and L. monocytogenes, the decrease was the highest in case of L. monocytogenes. In case of S. Enteritidis, the reduction was approximately 0.4 magnitude. Furthermore, significant difference can also be observed in the physical properties of the two samples. The similar test of pressure treatment time (Tests 3 and 9) also demonstrated differences in microbiological results; however, no definite conclusions could be drawn from these results. For example, reduction of L. monocytogenes count was not enhanced by the increased treatment time.

Table 2. Regression coefficients of the secondary polynomial model for response analysis with encoded units.

	$\lg(N/N_0)$, (CFU/mL)						Total viable cell count (N) (CFU/mL)	
	S. enteritidis		*L. monocytogenes*		*S. aureus*			
	β coefficient	p	β coefficient	p	β coefficient	p	β coefficient	p
Constant	5.192	0.00	1.583	0.00	1.965	0.00	2.315	0.00
P	0.005	0.03	0.006	0.00	0.004	0.05	−0.005	0.00
T	−0.017	0.49	0.010	0.24	0.055	0.06	−0.014	0.06
$P \times T$	0.010	0.95	−0.004	0.93	−0.010	0.94	−0.057	0.13
P^2	−0.055	0.64	−0.064	0.13	0.157	0.18	−0.168	0.00
T^2	0.336	0.03	−0.023	0.55	−0.097	0.38	−0.012	0.68
$M(r^2)$	0.89		0.98		0.88		0.99	

By evaluating the secondary polynomial model created for our test results, the effects of each variable can also be mathematically analysed. It is clearly seen that most of the models fit relatively well to the test result; r2 did not exceed 0.9 only for the testing of reduction of S. Enteritidis and S. aureus count. However, the correlation of measured and calculated values were very high for reduction of L. monocytogenes viable cell count (r2 = 0.98), total viable cell count after treatment (r2 = 0.99) and viscosity (r2 = 0.99).

Analysis of p-values for various β coefficients demonstrates that destruction of various microorganisms was significantly ($p < 0.05$) affected only by the pressure level. Extent of colour change of samples was similarly affected by the pressure level only. However, treatment pressure level and time both significantly affected the increase in viscosity.

By using these models, we can measure the approximate microbiological and physical changes in liquid whole egg in the experimental range with the specified treatment parameters. Graphical illustration of models clearly shows the effect of each parameter. Figure 1 shows the reduction of L. monocytogenes viable cell count in liquid whole egg induced by HHP treatment based on the model calculated in function of the treatment pressure level and time. It can be observed from the figure that the pressure affected the reduction of L. monocytogenes viable cell count.

Figure 2 shows the measured and calculated results for the model. The high correlation between the measured and calculated results can be clearly seen as given in Table 2.

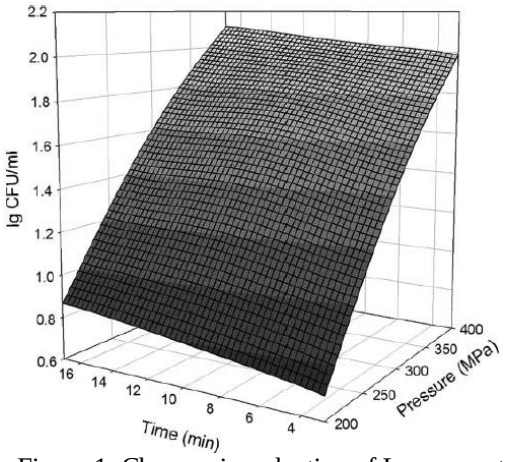

Figure 1: Changes in reduction of L. monocytogenes viable cell count in function of treatment pressure and treatment time.

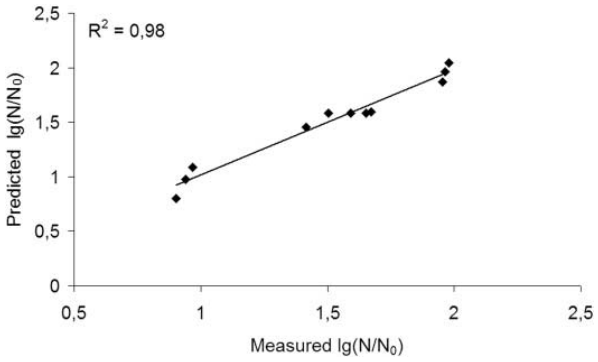

Figure 2: Correlation between the model for reduction of L. monocytogenes viable cell count and the measured results.

4. CONCLUSIONS

In each test of our investigations, the viable cell count of S. Enteritidis critical for egg products was reduced significantly (at a level of 4.5 magnitude or above), which correlates well with the literature data, whereas the reduction of the total viable cell count was around two magnitudes.

In our studies, we obtained models showing good correlation with our measured results. In conclusion of these results, microbial destruction and colour change caused by the treatment at HHP are primarily affected by the pressure level, while the changes in viscosity are also significantly affected by the duration of high pressure treatment ($p < 0.05$).

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WASTEWATER TREATMENT FROM THE EDIBLE OIL INDUSTRY BY TUBULAR CERAMIC MEMBRANE

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SUMMARY

Membrane technology is convenient for separation of suspended solids, colloids and high molecular weight materials that are present. The idea is to microfilter the wastewater of the edible oil industry, after the separation of oil by skimmer, where the permeate passes through the membrane and are available for recycling, and can be returned to the process.

For the microfiltration the wastewater from edible oil industry were used. For microfiltration of this effluent a tubular membrane was used with a pore size of 200 nm at transmembrane pressure in range up to 3 bar and in range of flow rate up to 300 l/h. The permeate chemical oxygen demand (COD) was reduced for 60% according to the feed. And the highest flux was 160 l/m^{2}h.

1. INTRODUCTION

Membrane separation is a filtration technique in which a feed stream is fractionized with a porous membrane. Some of the dissolved solids are held back because their molecular size is too large to allow them to pass through. The size range depends upon the pore sizes of the used membrane. Fractionation of the feed stream occurs, with some molecules being concentrated on the upstream side of the membrane, which is known as the concentrate or retentate. The smaller molecules pass through the membrane into the permeate stream. The driving forces that cause mass transfer of solutes are usualy difference in concentration, difference in electric potential, difference in pressure (Šereš, 2009).

The main problem in the performance of microfiltration is concentration polarization and fouling of the membrane. Concentration polarization causes deposition of retained compounds on the membrane surface. The pure water flux of micro- and ultrafiltration membranes is usually high, but when separation starts through the membrane, the permeate flux falls very quickly, which is caused by the gel formation on the membrane surface. Membrane fouling is a sedimentation or accumulation of suspended or colloidal particles on the membrane surface, as well as crystallization, precipitation or adsorption of solute on the surface and / or in the pores of the membrane. There is no possibility for avoiding membrane fouling, but it can be limited by applying a number of different techniques which enhance membrane flux. These techniques might be pre-treatment of feed stream, backflushing, fluidized bed, fluid instability, application of electric, magnetic and ultrasonic fields (EC, 2003; Šereš, 2009).

This paper will discuss the possibility of applying the new generation of ceramic membranes in order to reduce the chemical oxygen demand (COD) of wastewater oil industry. The process would result in a permeate water with reduced COD and no turbidity, which can be recirculated into the process, and it should be possible to apply the retentate (concentrate) to wood dust and thereby improve its energy value. During te production of edible oil is produced wastewater of 10-25 m^3/t of product, with specific production wastewater 3-5 m^3/t of raw material.

According to the Regulation on limit values of emissions of industrial waste water, which is discharged into the public sewer, limits, among other things, for the chemical oxygen demand (COD) is 1000 mgO_2/l , biochemical oxygen demand (BOD) - 500 mg O_2/ l (Dalmacija, 2011).

2. MATERIALS AND METHODS

In this work for the experiments was used pooled effluent oil industry. Characteristics of this wastewater are: chemical oxygen demand in the range of 5000-18000 mgO_2/l, turbidity in the range of 200-2500 NTU.

In order to remove coarse contaminants before the microfiltration, wastewater was filtered through the cotton cloth. Installation for micro and ultrafiltration, which is used in this paper was designed at the Faculty of Technology in Novi Sad. Scheme of laboratory plant for micro- and ultrafiltration is shown in Figure 1.

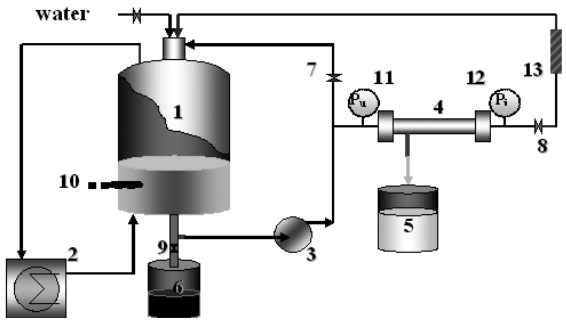

Figure 1: 1 – tank, 2 – termostat, 3 – pump, 4 – module with membrane,
5 – tank for permeate, 6 – tank for retentate, 7 – ventil for flow rate correction, 8 – ventil for pressure correction,
9 – ventil for retentate, 10 – termometer, 11 – manometer,
12 – manometer, 13 – rotameter

For the purpose of this study,a ceramic membrane is used, manufacturer GEA with pore opening size of 200 nm. Membrane is tubular shaped, length 250 mm, with an outside diameter of 10 mm and an internal diameter of 6.8 mm. The experiments of microfiltration of wastewater are planned based on the Box-Behnken's plan. Table 1. shows the values of independent parameters that were varied (Jokić, 2010).

Table 1. Varied values of independent variables

Parameters	Q [L/h]	T [^oC]	P [bar]
Varied values	100 / 200 / 300	20 / 50 / 60	1 / 2 / 3

Q - flow [l / h]
T - Operating temperature [° C]
P - transmembrane pressure [bar]

Dependent parameter which is constantly monitored during the process of is the permeate flux:

$$J = \frac{V_p}{A_m \cdot t} \quad (1)$$

J - permeate flux [L/m^2h], V_p - volume of permeate [L], A_m - membrane surface [m^2], time of microfiltration [h]

Physico-chemical parameters such as chemical oxygen demand and turbidity in the sample, permeate and retentate were also measured. Chemical oxygen demand (COD) is determined by the titrimetric method SRPS ISO 6060 (Official Gazette of FRY, no. 45/94,

1994). Turbidity is determined by the device Turb 550 IR. The measurements are performed automatically.

3. RESULTS

Before examining the conditions of filtration of wastewater, it is necessary to determine the dependence of the flux of distilled water from the transmembrane pressure for the possibility to compare with the results of permeate flux of wastewater. In Figure 2 is presented the dependence of the flux of distilled water and the permeate flux of wastewater. Flux of water is the basis for comparison, since it in plays the role of the solvent in wastewater. It shows that the the flux of distilled water is from 5 to 8 times greater than the flux of permeate, indicating the additional resistance to the flow through the membrane pores during microfiltration of wastewater.

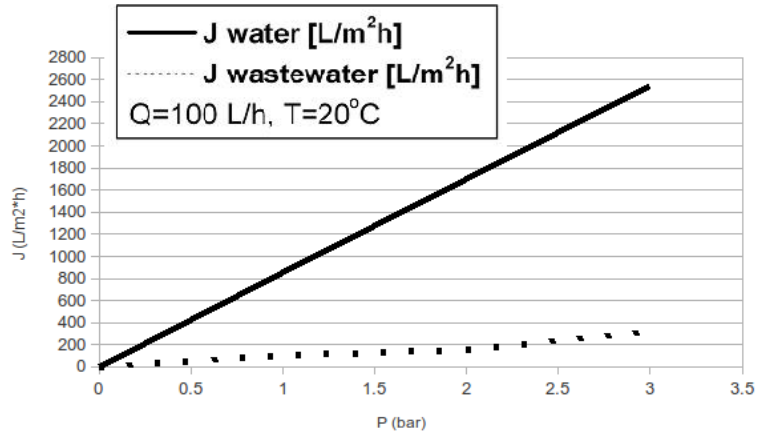

Figure 2. Water flux and wastewater permeate flux, depending on the transmembrane pressure at microfiltration on ceramic membrane

On the basis of experimental values graph of the two dependencies is drawn and by the program Statistica 12 the values of such get the equation that best describes the function of the flux dependence on pressure and flow. The highest values of flux, above 160 L/m^{2}h, are obtained when the flow is maintained above 200 L/h and pressure tested at higher values (above 2 bar). In Figure 3 are given values of chemical oxygen demand (COD), and turbidity (turbidity) of wastewater before and after microfiltration microfiltration in permeate and retentate.

Figure 3 shows that by using membranes of 200 nm chemical oxygen demand is decreased by 85% and by 99% for turbidity.

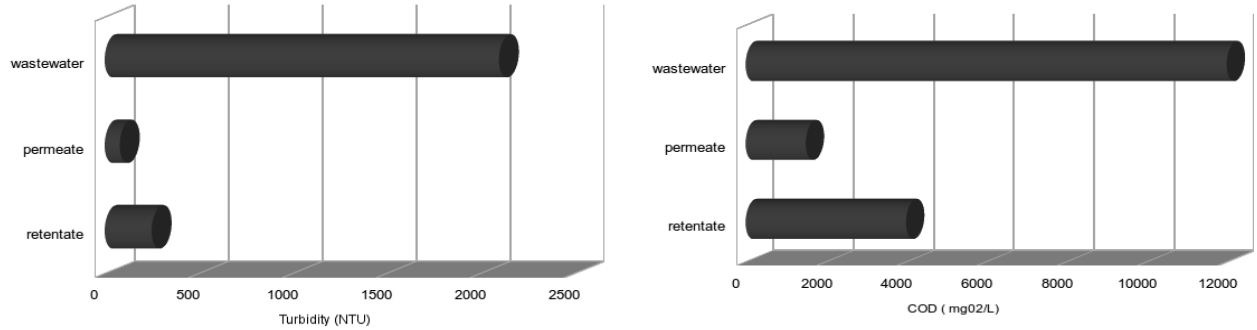

Figure 3. Chemical oxigen demand (COD) and turbidity before microfiltration in wastewater and after microfiltration in permeate - purified wastewater, and retentate

4. CONCLUSION

Based on the effects of microfiltration of wastewater at a transmembrane pressure in the range of 1 - 3 bar, flow rate of 100 to 300 L/h and temperatures of 20 - 60°C, it can be concluded that microfiltration can reduce the chemical oxygen demand of waste water, ie. contamination of waste water:

- Permeate flux wastewater (95 L/m2h) at a transmembrane pressure of 1 bar is 8 times smaller than the flux of water and the trend retains on rising pressure. That leads to the formation of resistance during microfiltration of wastewater on the surface membrane from compounds present in the wastewater.
- At pressures above 2 bar can be achieved permeate flux of 160 L/m2h.
- Using membranes from 200 nm to reduce the chemical oxygen demand by 85%, while the turbidity removes up to 99% of the waste water

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APPLICATION OF HIGH HYDROSTATIC PRESSURE TO EXTEND THE SHELF-LIFE OF FRESH MEAT

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SUMMARY

The aim of the examinations was to define an optimal pressure range in the use of high hydrostatic pressure on fresh meat, where denaturation of proteins and myoglobin is not significant, so it doesn't cause imperfect colour, but it adequately inhibitsthe multiplication of microorganisms. The focus of the measurements was how the different pressures influence the physical, organoleptic and chemical features of fresh meat. The colour of the meat changed only slightly during the 200 MPa pressure treatment, the difference between the colour of the untreated and the treated samples was noticeable only when laid side by side. As a result of treatments at 400 or 600 MPa the colour of the meat became lighter on both the internal and external surface. After the treatment the samples could not be distinguished by their colour. On the basis of the results the following conclusion could be made: the high hydrostatic pressure treatment didn't influence significantly the pH of the meat. After the pressure treatment there was only 2-3% liquid loss. According to the microbiological results the treatment lowered the original number of germs, so the shelf life of the meat increases without significantly damaging the quality of the meat.

1. INTRODUCTION

The HHP, High Hydrostatic Pressure, as a mild, non-thermal preservation technique has spread all over the world in the food industry since the year 2000 because of its numerous beneficial characteristics.

One of the most important of them is that HHP inactivates pathogens and vegetative spoilage microorganisms by using pressure rather than heat to effect pasteurisation. HHP utilizes intense pressure (about 400-600 MPa) at chilled or mild process temperatures, allowing most foods to be preserved with minimal effects on taste, texture, appearance or nutritional value (Balasubramaniam et al., 2011). These favourable characteristics make HHP suitable to be used in a wider range especially in the meat industry.

However, foregoing experiments showed that high pressure treatment of myoglobin caused partial denaturation, but the process was reversible (Defaye et al., 1995).

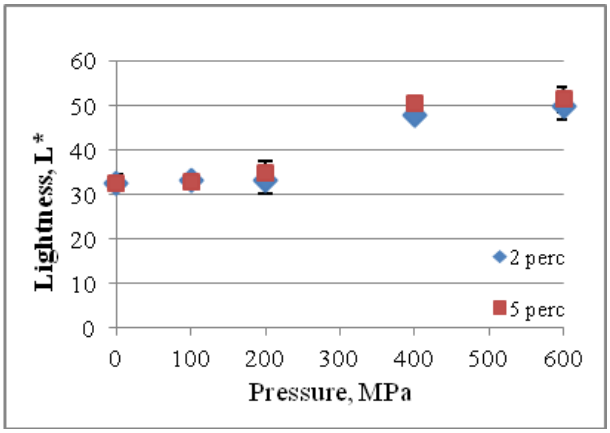
Murano et al. (1999) obtained a 10-$\log_{10}$ reduction in the number of the most resistant strain of *L. monocytogenes* in fresh pork sausage with a treatment of 400 MPa at 50 °C for 6 minutes. The efficacy of treatment against spoilage microorganisms resulted in a shelf life extension of 23 days in storage at 4 °C with no substantial impact on the sensory qualities (Campus, 2010).

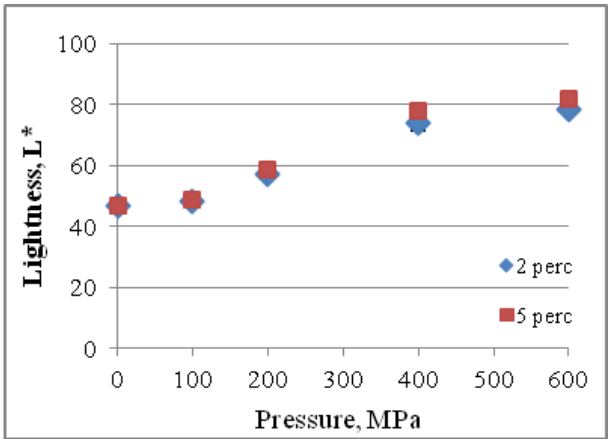
2. MATERIALS AND METHODS

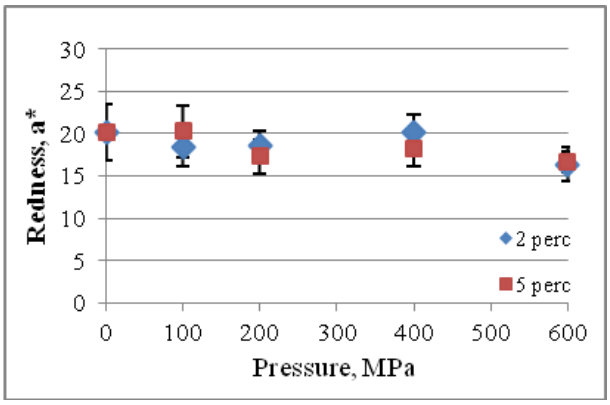
During the first experiment, slices of chicken breast and beef silverside (semitendinosus muscle) were pressurized at *100, 200, 400* and *600 MPa* for *2* and *5 minutes, respectively*. The optimal parameters for the second experiment were chosen from the values of the first one: *200 MPa* for *5 minutes*.

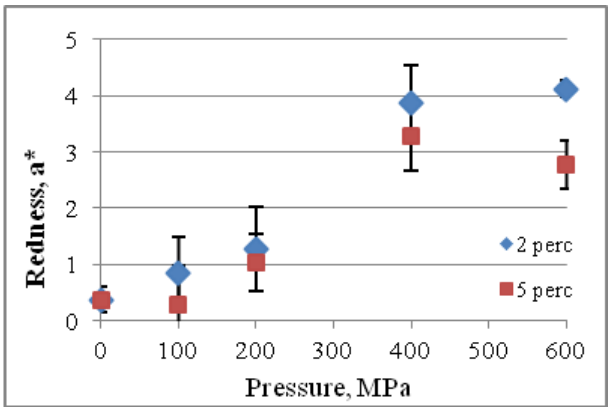
The treatments were accomplished with a Resato FPU-100-2000 high-pressure processing system. The colour measurements were carried out with a Minolta CR-200 tristimulus colorimeter. The pH, the drip loss of the meat and the temperature of the chamber during the treatment were also measured. After the treatment the unpacked samples were stored for 5 days and the vacuum packed samples were stored for 22 days between 0-5 °C. Aerobic plate counts of samples were also determined.

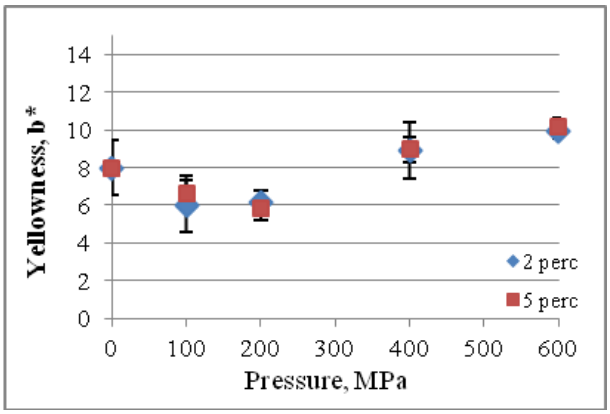
3. RESULTS AND DISCUSSION











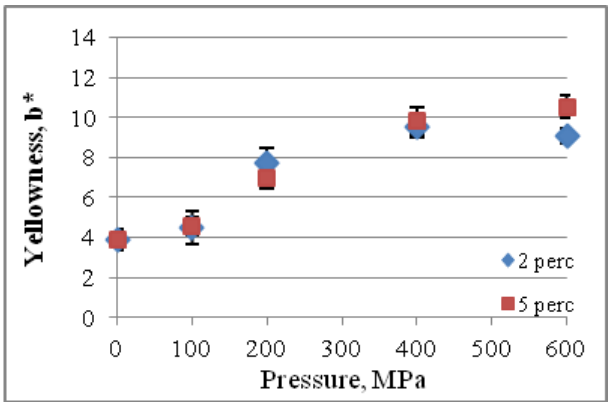


Figure 1: L* a*b* parameters of beef meat after HHP

Figure 2: L*a*b* parameters of chicken breast meat after HHP

The lightness values (L*) of both types of meat were growing by the increase of pressure. Redness (a*) and yellowness (b*) of the treated samples also shifted from the values of the control samples. Discolouration of the meat increased apparently above the pressure of 200 MPa. This alteration was caused by the partial denaturation of myoglobin and the oxidation of ferrous myoglobin.

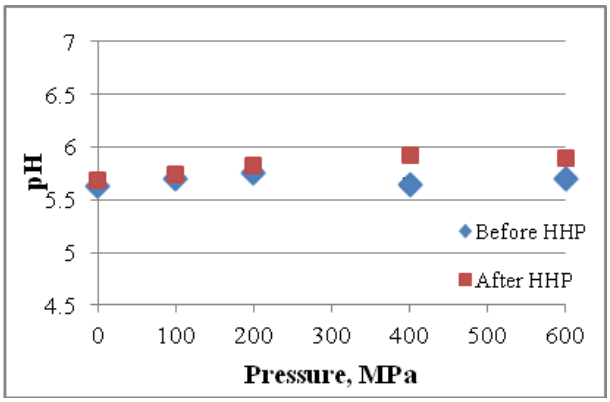


Figure 3: Effect of HHP on the pH of beef meat

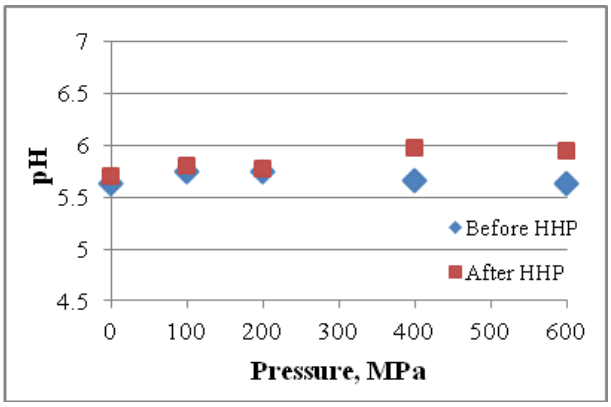


Figure 4: Effect of HHP on the pH of chicken breast meat

Increasing the applied pressure, pH of the meat samples slightly increased, but the pH of the untreated samples were below the level of the treated ones. After all, the high hydrostatic pressure treatment didn't influence the pH of the meat significantly.

The results of the mass measurement showed that only a maximum of 2-3% drip loss was caused by the pressure treatment.

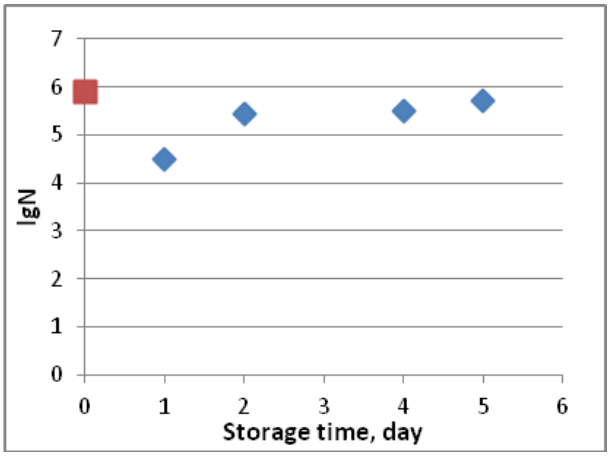


Figure 5: Number of cells on a beef meat stored for 5 days without packaging

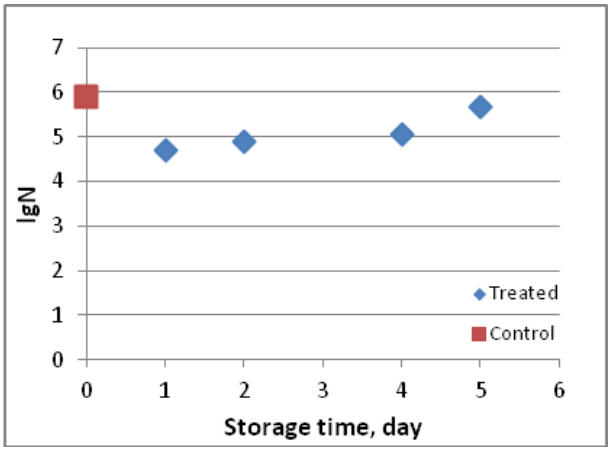


Figure 6: Number of cells on a chicken breast meat stored for 5 days without packaging

The limit value of the cells on the surface of the fresh meat was 10^7 cfu/g. Both types of meat had 10^6 cfu/g cells on their surface before the 200 MPa HHP treatment for 5 minutes. The treatment caused a two log cycle decrease. At the end of the 5-day-storage the number of viable cells did not reach the limit on either type of meat.

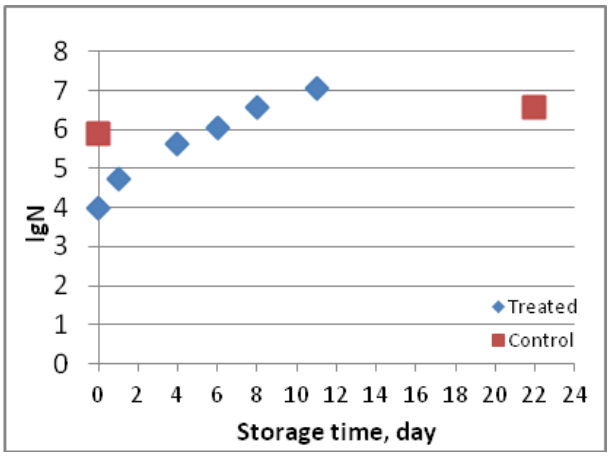


Figure 7: Number of cells on a beef meat stored for 22 days in vacuum packaging

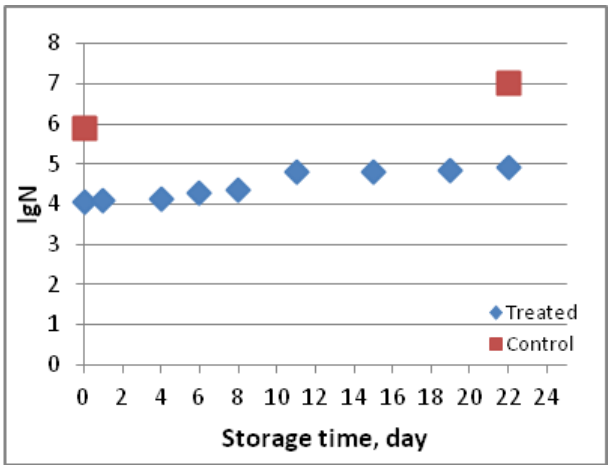


Figure 8: Number of cells on a chicken breast stored for 22 days in vacuum packaging

At the end of the 22-day-storage the number of cells reached the original 10^6 cfu/g, but still remained below the number of the cells on the control samples. The results of the experiments confirmed, that the shelf life of the treated samples was longer than the shelf life of the untreated ones.

4. CONCLUSIONS

These experiments have led to the conclusion that HHP can be adopted in three sections of the food industry:

- Catering/restaurants
- Manufacturing meat products
- Fresh meat counters in supermarkets

In case of the first and second sectors higher pressure can be applied to reach a longer shelf life, which results in discolouration, but it has no significance for the final consumer because it is not perceptible after the processing. In case of the third sector a lower pressurization should be applied to get an increased shelf life without a significant loss of colour.

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EXAMINATION OF DRYING TEMPERATURE DEPANDING POLYPHENOL-OXDASE ENZYME ACTIVITY AND COLOUR CHANGES OF DIFFERENT COMPOSITION DRY PASTA PRODUCTS

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SUMMARY
Due to the favourable colour characteristics and nutritional values of millet (Panicum miliaceum L.*), it may be a suitable material for high quality pasta products without egg. Because of the millet does not contain gluten, making the appropriate structure of dough adding other flours or additives are recommended.*
During the author's work, equal amount of millet was added to different flours (Triticum aestivum L., *Triticum durum* L.*) in the manufacture of the studied pasta products. The industrial usability, polyphenol-oxidase enzyme activity, the moisture content and the colour changes of millet pasta was studied using different drying temperatures, which may cause significant affects during the manufacture of dry pasta products.*

1. INTRODUCTION

Improving the nutritional values and the colour of dry pasta products is one the most common research and development topic. Addition of millet (*Panicum miliaceum* L.) has a number of positive effects on the pasta: increases the level of antioxidants, minerals (magnesium, iron, calcium, fluoride, silicon) and vitamin B1-B2-B6 content, and reduces the *GI* (glycemic index) of the final product (Cubbada et al., 2009, Shaidi and Chandrasekara, 2013). On the other hand, due to the favorable colour characteristics of millet a better preferred product can be produced without the addition of eggs, thus getting a microbiologically safer dry pasta product. However, the polyphenol oxidase enzyme (PPO) level is significantly increasing by adding millet to the pasta (Tolbert, 1973). The presence of the PPO enzyme is undesirable in pasta products, because the yellow colour of the pasta is significantly compromised by the oxidation of phenoloids. This enzymatic browning can be prevented by the sufficiently high drying temperature (Svec et al., 2008). However, it is important to avoid the usage of high drying temperature (> 800C), which is very common in the dry pasta industry, because it can cause damage in the levels of vitamins, antioxidants and colour agents added by the millet.

2. MATERIALS AND METHODS

The moisture content of the pasta samples was determined by the Sartorius device, using five replicates per sample. The drying process was performed by ARMFIELD fluid dryer.
The colour characteristics were studied by Minolta CR-130 Chromameter. From the colour parameters the drying temperature depending L* and b* values were considered. The higher b* values show more yellow tone of the pasta, while the L* values refer to the clarity.
The PPO activity was measured by using synthetic substrate, pyrocatechol. The oxidized form of the substrate can be determined by photometrically at 420 nm by spectrophotometer (Watson és Flurkey, 1986).

The sensory analysis was based on the current standard, on the Codex Alimentarius Hungaricus 2-85. The pastas can be divided into 3 groups based on their scores: class I. (20-16 points), class II. (15-12 points) and class III (<11.9 points).

All analyses were performed at least in quintuplicate. Standard deviation was within ±5%.The codes and the compositions of the different pasta samples are shown in **Table 1**.

Table 1: Pasta sample codes and compositions

Number of samples	Composition of samples
1	***Triticum durum* L. flour (*T. durum*)**
2	***Triticum aestivum* L. flour (*T. aestivum*)**
3	**70% Durum + 30% Millet flour**
4	**70% TL-50 + 30% Millet flour**

3. RESULTS AND DISCUSSION

The measurement of the different pasta samples using the same temperature but at different drying time resulted what we expected: the moisture content of the pasta samples was significantly reduced in all cases caused by the longer term drying process, however, we could not achieve 13% moisture content by ten minutes long drying term (**Figure 1**). Based on the results, we can say, that with the increasing drying temperature the effect of the addition of millet decreased more and more.

The PPO activity was typically higher in the millet content pasta samples, which corresponds to the prior expectations. We could achieve significant reduction of the PPO activity in the millet containing pasta samples by drying at 70 ^{0}C, but the PPO activity values measured at this times were four times higher compared to the only *T. durum* containing pasta samples. The measurement data also shown, that the PPO activity of T. durum-millet mixture containing pasta samples decreased in greater extent than the *T. aestivum* and millet mixture pasta samples. The biggest difference between the millet containing mixtures was detected at 60 ^{0}C drying temperature (**Figure 2**).

The examination of colour changes shows, that the short-term, low-temperature drying process did not cause significant changes in the L* values (**Table 2**). The yellow colour intensity of the *T. durum* pasta was decreased, while the *T. aestivum* pasta sample was improved with addition of millet. Matching the preliminary expectations, the b* values of the samples decreased when the drying temperature was increasing. The most favourable b* value was measured in the *T. durum* pasta sample, which could be approached only with the *T. aestivum* and millet mixture. We found no unequivocal relationship between the PPO activity values and the colour characteristics.

Based on the result of the sensory test (**Table 3**), we can conclude, that the 100% *T. durum* made pasta got the highest score, followed by the mixture of millet and *T. durum* sample. These samples are qualified as first class pasta by the obtained points. The *T. aestivum*, *T. aestivum* and millet mixture pasta samples reached significantly lower points. The received scores shows that enrichment of *T. durum* with millet flour did not affect significantly the flavour of the product, and a fine, colour balanced and preferred pasta product can be manufactured. The sensory evaluators did not prefer the *T. aestivum* made pasta sample, which did not change with the millet enrichment. It is also seen, that not any of the flours should be enriched with millet or other grist, so further experiments, measurements and sensory qualifications are required.

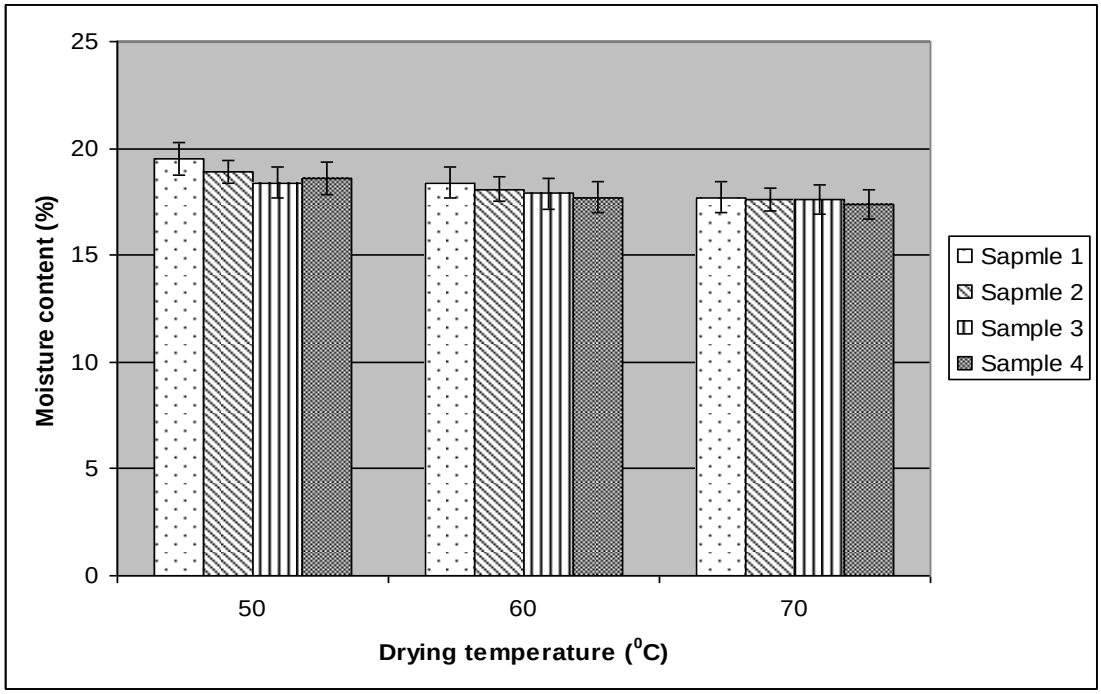

Figure 1: Changes in moisture content during drying

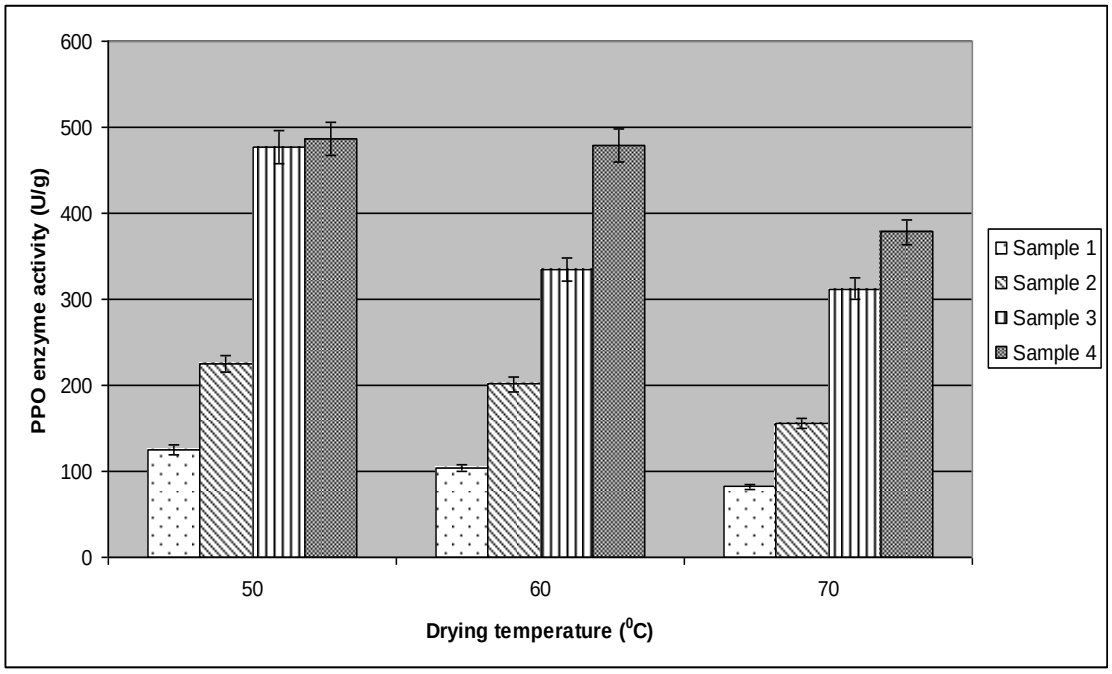

Figure 2: PPO enzyme activity after drying

Table 2: Colour changes in pasta samples at different drying temperatures

Sample	Parameter	50 ^{0}C	60 ^{0}C	70 ^{0}C
1	L*	98.4	99.5	98.7
	b*	1.66	1.62	1.58
2	L*	104.2	104.6	10.,5
	b*	-10.5	-10.2	-10.1
3	L*	9.1	99.3	97.5
	b*	-2.8	-3.3	-3.5
4	L*	103.2	102.8	98.3
	b*	1.38	1.21	0.7

Table 3: Result of sensory analysis

Sample	Score	Class
1	19	I.
2	16	I.
3	18	I.
4	15	II.

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QUALITY AND SHELF-LIFE DETERMINATION OF SWEET PEPPER VARIETES BY NONDESTRUCTIVE METHODS

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SUMMARY
All the nowadays available pepper varieties are characterised by a relatively short shelf-life due to their susceptibility to fast quality decrease. In order to monitor the quality changes and compare the varieties nondestructive texture analysis, tristimulus colour measurement and chlorophyll-fluorescence analysis were carried out during storage at room temperature and combined with cold storage in case of 5 different pepper varieties (3 sweet: Kárpia, Carma, Gigant, 2 hot: Kais, Kun). According to our results, in case of mass loss, stiffness, surface colour and chlorophyll fluorescence changes, the Gigant, Carma and Kárpia varieties were found to be favourable, but in contrast, hot pepper samples of Kais and Kun were found to be the most sensitive to negative quality changes concerning shelf-life. In general, the shelf-life storage combined with prior cold storage resulted in a relatively longer shelf-life, with a lower intensity and rate of quality decrease in time, taking into account the results of mass loss, acoustic stiffness measurment and chlorophyll fluorescence analysis.

1. INTRODUCTION

The commercial value and the marketability of fresh horticultural products are determined by their quality, storage and shelf-life properties. Sweet pepper belongs to the really sensitive vegetables to rapid negative quality changes with relatively short shelf-life among improper postharvest conditions. Due to the application of advanced transportation techniques, facilities and packaging methods, the less efficient called shelf-life period could be characterised by a higher risk of quality loss and lower possible commercial value. According to the high demand for fresh products, there is a continuous need for the investigation of postharvest properties of sweet pepper too. Rapid and non-destructive quality determination methods such as acoustic stiffness and impact firmness measurement, chlorophyll fluorescence analysis, image processing methods, are frequently used in quality determination and characterization of fruit responses (e.g. physiological status, stage of maturation) to different external stressors, to quantify or predict produce quality (DeEll et al., 1999, De Ketelaere et al., 2006; Diezma-Iglesias et al., 2006; Kosson, 2003), but sweet pepper's objective quality determination is not an easy task to solve.

2. MATERIALS AND METHODS

Fresh and in optimal stage of maturity harvested 150 pieces of 5 different pepper varieties (3 sweet: *Capsicum annuum L. cv. Kárpia [red]*, *cv. Carma [pale yellow]*, *cv. Gigant [dark green]*, 2 hot: *cv. Kais* and *cv. Kun [dark and pale green]*) were measured in our experiments. For the simulation of the average commercial circumstances, the K10 marked samples (15 samples per variety) were cold stored at +10±1 °C (above the threshold temperature for chilling injury) for a week in a commercially available, but temperature controlled refrigerator and thereafter withdrawn for shelf-life storage at room temperature (+20±1 °C) without any packaging. The K20 marked samples (control samples) were stored continuously for the same time period at room temperature. Postharvest changes were measured and determined by the non-destructive acoustic impulse response measurement, chlorophyll fluorescence analysis, tristimulus surface colour measurement, calculation of average mass loss and by the evaluation of visible changes and defects. Measurements were

carried out at the beginning of the experiment and subsequently in every second or third day during shelf-life.

Chlorophyll fluorescence measurements were carried out by the use of a PAM WinControl-3 controlled MONI-PAM chlorophyll fluorometer (Heinz Walz GmbH, Germany) in order to determine the change in photosynthetic activity (closely related to the tissue's photosynthetically active chlorophyll content). Minimum and maximum fluorescence (F_0, F_m) were measured. Variable fluorescence ($F_v=F_m-F_0$) and maximum photochemical efficiency (F_v/F_m) were calculated. Measurements were carried out at the two directly opposite sides of each sample at the equatorial part.

CIE1976 L*, a* and b* absolute surface colour coordinates were measured at the same points of chlorophyll fluorescence analysis using a Minolta CR-200 compact tristimulus colour analyser (Konica Minolta Inc., Japan).

In case of acoustic impulse response technique (Felföldi, 1996) the vertically oriented pepper samples were tapped lightly on the top end with a wooden stick. The acoustic response was collected by a microphone located under the cushioning sample holder and recorded by a sound card in a PC. Custom Fast Fourier Transform software was used to analyse the recorded acoustic response. The characteristic frequency and the sample mass were used to calculate the acoustic stiffness coefficient: $S = f^2*m*10^{-6}$ [N/mm], where f is the characteristic (peak) frequency of the sample [Hz] and m is sample mass [g]. For the calculation of the S values and average mass loss (% of initial fresh weight) a Scaltec SAC 72 laboratory scale (Scaltec Instruments GmbH, Germany) with 1 g accuracy was used.

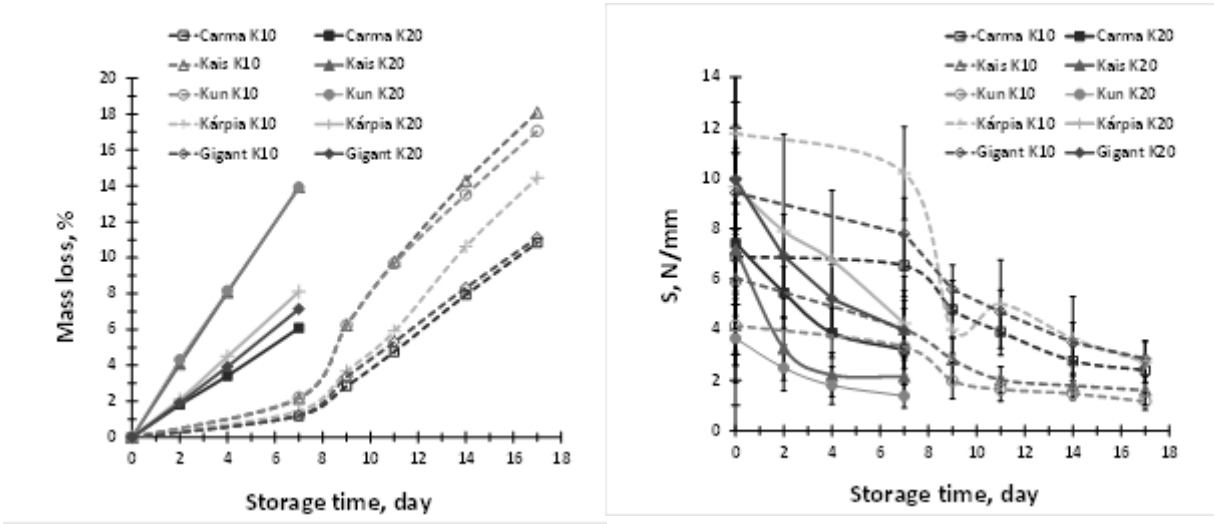
For data conversion MS-Excel and for statistical analysis SPSS ver.14 were used at 95% confidence level.

3. RESULTS

In contrast to the only minor negative changes of the samples stored at +10 °C (K10) for a week, the disadvantageous effect of the shelf storage at 20 °C became clearly visible after few days as variety dependent surface coloration (Gigant, Kais and Kun), as wilting, shrinkage and softening (Kais and Kun). After withdrawal of the good overall quality featured samples from 10 °C, these samples also suffered during shelf-life not preferable colour, mass and texture changes as the K20 samples, resulting in an unacceptable quality at the 17th day.

The mass loss of the samples during cold storage at 10 °C - independently from variety – was under 2 % due to the positive effects of low temperature on keeping quality, but after withdrawal to shelf life conditions, the mass loss increased rapidly (Fig.1A). The hot varieties (dark green Kais, light green Kun) suffered the highest mass loss during shelf-life and entire period, resulting in softening, wilting and finally a not acceptable overall quality in contrast to the advantageous results of the sweet varieties (Carma, Kárpia, Gigant), which were found to be the best concerning shelf-life. In Fig.1A the average mass loss of the K10 marked samples (cold and shelf stored ones) during the 14 days of combined storage was only a little more than the K20 marked samples during the one week of shelf storage.

The textural changes determined as acoustic stiffness coefficient change, clearly proved the results of the mass loss changes. Fig.1B shows the clear effect of cold and shelf storage on stiffness, as only minor textural decrease was measured in case of all varieties during the cold period. At 20 °C stored samples suffered rapid and significant stiffness decrease during the first 2-3 days resulting in the softening phenomena. Compared to this, the observed acoustic stiffness decrease during the first 2-3 days after withdrawal to shelf-life conditions was found to be less severe and intensive in time, except of Kárpia variety, but all the samples suffered significant decrease in stiffness and in overall quality.

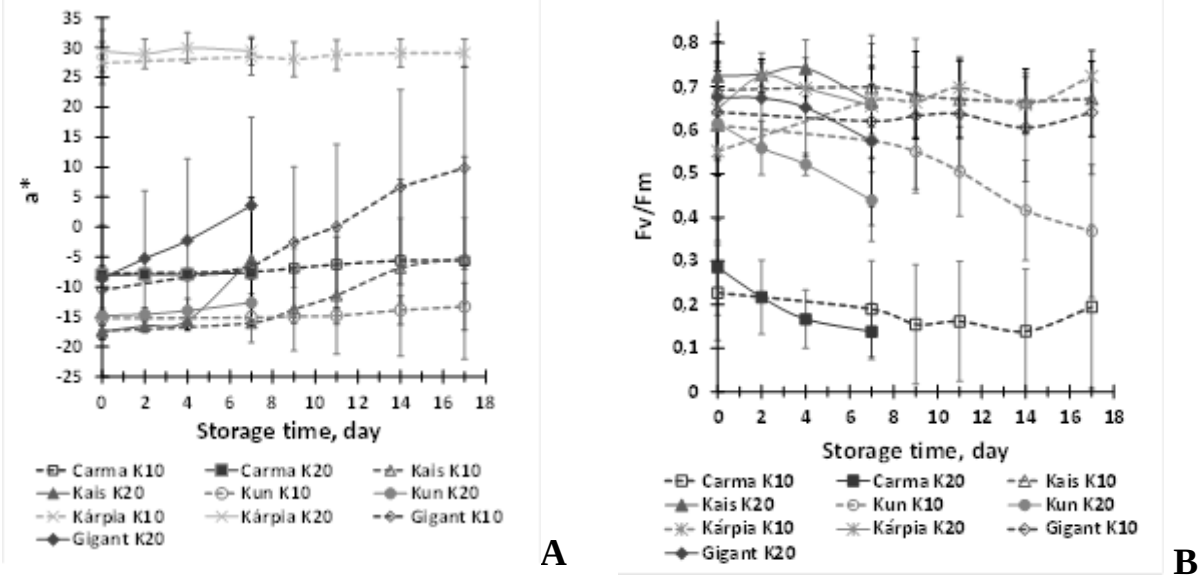


A B

Figure 1: Mass loss (A) and acoustic stiffness coefficient (B) change of pepper varieties vs. storage time

In case of the surface colour measurement, the post-colouration was clearly visible concerning the K20 marked samples after 5 days of shelf storage, except of the almost mature red coloured Kárpia samples (Fig.2A). In case of the Carma and Kárpia samples stored at 10 °C for one week (K10), no significant colour change was observed in contrast to a minor change of the mature green varieties (Kais, Kun and the blocky type Gigant). Except of the Gigant, the L* (not shown) colour parameter showed no significant change during postmaturation. Except of the Kárpia samples, concerning the a* parameter's (from green to red) change of the K10 and K20 samples stored at 20 °C, a variety and temperature dependent intensity characterized continuous a* increase was measured referring to an inhomogeneous maturation pattern only measurable with great standard deviation.

Chlorophyll fluorescence measurements also proved the earlier statements. No significant difference was found in case of the measured (F_0, F_m not shown) and calculated (F_v/F_m) initial parameters except the mature red Kárpia, but all parameters showed variety characteristic decrease during shelf storage (Fig. 2B) as the inhomogeneous maturation took place.



A B

Figure 2: a* colour coordinate (A) and the photosynthetic activity (B) change of pepper varieties vs. storage time

The measured fluorescence changes (decrease in F_m and F_v/F_m) clearly represented in case of the green varieties (Kun, Kais, Gigant, Carma) the temperature dependent, variety characteristic and photosynthetically active chlorophyll content related maturity and color change, either during cold or shelf-life storage at simulated retail conditions, but with different kind of intensity. The advantageous effect of lower temperature is obvious, taking into consideration the change of the fluorescence parameters (F_0, F_m and F_v/F_m), as the fluorescence changes are less intensive in time in case of samples stored at 10 °C prior to the shelf life storage at 20 °C compared to the normal storage results at 20 °C. In coincidence with Zsom et al. (2010), the rate of the photosynthetically active chlorophyll content related change of Fv/Fm was low in case of the red Kárpia, but proved the measureable presence of photosynthetically active chlorophyll content even in the case of mature red stage of ripeness.

4. CONCLUSIONS AND SUGGESTIONS

According to our result, all the used nondestructive measuring methods were found to be suitable for the detection of postharvest quality changes in case of the different pepper varieties. Concerning the behavior during cold storage and subsequent shelf storage simulating the possible commercial conditions, upon the data of overall quality, mass loss, postcolouration, textural degradation the dark and pale green hot varieties (Kais, Kun) were found to be as less shelf-life suitable ones in contrast to the green blocky type Gigant, the pale greenish yellow Carma and the red Kárpia. Due to the positive effect of low but not chilling temperature, combined cold and shelf storage was found to be useful for an in time less intensive overall quality decrease during shelf life. The majority of the observed mass and texture decrease occurred rapidly during the very first days even after withdrawal to shelf-life conditions from cold storage too, resulting in a lower overall quality and commercial value.

Concerning the variety dependent results of surface colour measurement and chlorophyll fluorescence analysis providing only local, but not overall information, the inhomogeneous maturation with increasing and overlapping standard deviation in time suggested the need for a higher number of sampling points, subsequent sampling at the significant colour changes and/or the use of machine vision and chlorophyll fluorescence imaging systems in order to determine colour and photosynthetic activity change during shelf-life postmaturation.

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MONITORING OF POSTHARVEST QUALITY CHANGES OF BOSC KOBAK AND CONFERENCE PEAR CULTIVARS BY NOVEL NONDESTRUCTIVE METHODS

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SUMMARY

*Postharvest changes of two different pear varieties (Pyrus communis cv. Bosc kobak and cv. Conference) at cold and shelf-life conditions were measured by non-destructive optical (chlorophyll fluorescence analysis, laser scattering and surface colour measurement) and texture analysis methods (acoustic impulse-response technique and impact method). The rate of the change of Fv/Fm was lower in case of Conference variety, referring to the variety characteristic and photosynthetically active chlorophyll content related maturity and colour change at both conditions with a different rate and intensity, as well as Hue° and L*a*b* parameters. The acoustic and impact stiffness coefficients decreased during the shelf-life, representing clearly the postharvest textural change closely related to temperature. Taking into account the seven different measuring wavelengths (650-1064 nm), the laser scattering parameters showed significant and cultivar dependent differences versus storage time during the cold storage and shelf storage period, separately.*

1. INTRODUCTION

Pears belong to the really perishable and sensitive fruits with relatively short shelf-life. Among improper storage conditions, disadvantageous internal and external changes can occur (i.e. texture, colour), affecting really negatively the storage potential and the possible commercial outcome. For the objective determination of postharvest quality changes of horticultural crops, non-destructive measuring methods (e.g. acoustic stiffness, impact firmness measurements, chlorophyll-fluorescence analysis, multispectral and backscattering imaging) are commonly used in postharvest technology providing information about the internal and external quality changes (Baranyai and Zude, 2009; De Ketelaere et al., 2006; Romano et al., 2008; Saquet and Streif, 2002, Taniwaki et al., 2009).

2. MATERIALS AND METHODS

120-120 samples of two different pear cultivars (*Pyrus communis cv. Bosc kobak and cv. Conference*) at optimal stage of ripeness were examined. Four times 30-30 samples were withdrawn from cooling chamber (2±1 °C) in two weeks intervals and were stored in 22±2 °C simulating the conventional shelf storage conditions for almost two weeks. Postharvest changes were measured by different non-destructive optical and texture analysis methods. After the initial measurements, the later ones were carried out at an almost daily basis on 30 pear samples per variety concerning with each withdrawal from cold storage and continued during shelf-life.

Chlorophyll fluorescence measurements were carried out by the use of a PAM WinControl-3 controlled MONI-PAM multi-channel chlorophyll fluorometer (Heinz Walz GmbH, Germany) in order to determine the change in photosynthetic activity (closely related to the tissue's photosynthetically active chlorophyll content). Minimum and maximum chlorophyll fluorescence (F_0, F_m) were measured. Variable fluorescence ($F_v=F_m-F_o$) and maximum photochemical efficiency (F_v/F_m) were calculated. Measurements were carried out at the two directly opposite sides of each sample at the equatorial part.

The dark box located laser backscattering imaging system is based on a high performance monochromatic CCD IP camera positioned vertically above the sample (Photon Focus MV1-

D1312, spectral sensitivity from 320nm to 1080 nm) and on seven solid-state laser diode modules emitting at seven spectral bands (532-1064 nm) set at 15° incident angle with almost the same focus point on the sample surface injecting the laser beams into the fruit tissue. The incident point surrounding surface area got illuminated by diffuse reflectance by into the fruit tissue entering laser beam. This illuminated area was scanned and intensity values were calculated with radial averaging relative to the incident point. Threshold of the 50% intensity level was used to segment illuminated area and background. From logarithm intensity profiles slope, inflection point (IP) and full width at half maximum (FWHM) were calculated.

ColorLite sph850 spectrophotometer (ColorLite GmbH, Germany) was used to scan the spectra from 400 to 700 nm with 10 nm steps by the detection of the light reflected from the samples' surface. XYZ, L*a*b*, L*uv and xy surface color parameters were recorded. Three repetitions were carried out per sample point located on both sides of the equatorial part of the samples (the same points as of chlorophyll fluorescence analysis).

In case of acoustic impulse response technique (Felföldi, 1996) pear samples were tapped lightly on the equator with a wooden stick. The acoustic response was collected by a microphone located under the cushioning sample holder and recorded by a sound card in a PC. Custom Fast Fourier Transform software was used to analyse the recorded acoustic response. The characteristic frequency and the sample mass were used to calculate the acoustic firmness coefficient: $S = f^2 * m^{2/3} * 10^{-6}$ [$Hz^2 g^{2/3}$], where f is the characteristic (peak) frequency of the sample [Hz] and m is sample mass [g].

The impact firmness measuring system consists of an impact hammer with a changeable mass and a built-in piezoelectric accelerometer, an electronic signal converter and a dynamic signal analyser recording and displaying the voltage signal of the accelerometer. Time and voltage differences between initial and maximum point of the curve were determined by a special program. The sample's firmness is characterised by the impact firmness coefficient (Felföldi and Ignát, 1999): $D = 1/\Delta T^2$ [ms^{-2}], where ΔT is the time difference between initial and maximum point of the curve [ms]. The average impact firmness was calculated by the data of the measured four points on the equator of each pear sample.

For data conversion MS-Excel and for statistical analysis SPSS ver.14 were used at 95% confidence level. The open source software of R (ver. 2.12.1, R Foundation for Statistical Computing, Austria) was used to perform statistical analysis, produce summary reports and charts in case of the laser scattering data analysis.

3. RESULTS

No significant difference was found in case of the measured (F_o, F_m, data not shown) and calculated (F_v/F_m) initial chlorophyll fluorescence parameters, but all parameters showed significant decrease during storage (Figure 1). The rate of the photosynthetically active chlorophyll content related change of F_v/F_m was lower in case of the Conference than the Bosc kobak variety. The measured fluorescence changes clearly represent the variety characteristic and photosynthetically active chlorophyll content related maturity and color change either during cold storage or during shelf-life storage at simulated retail conditions, but with different kind of intensity.

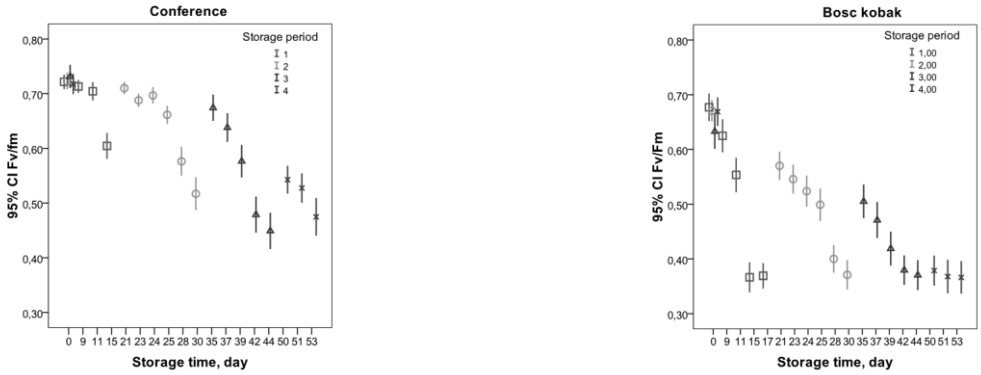

Figure 1: The change of the Fv/Fm chlorophyll fluorescence characteristic of the pear samples vs. storage time

The Hue° and L*a*b* surface colour parameters clearly and significantly showed the different rate of the colour change during the cold and shelf storage period. Fig. 2 shows the a* colour component's change. The initial colour of the two pear variety was found to be significantly different (dark green Conference and yellowish green Bosc kobak samples).

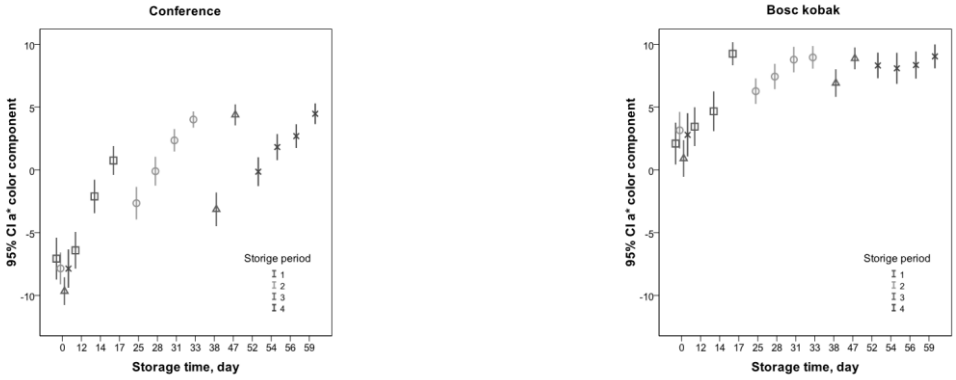

Figure 2: The changes of the pear varieties' a* colour component vs. storage time

The a* and Hue° values of both varieties (Hue° data are not shown) reflected clearly the change from green to yellow colour representing the effect of storage temperature on ripening related surface colour change as the samples' colour changed slowly at low temperature compared to the really fast change at the higher room temperature during shelf-life.

The impact stiffness coefficient decreased drastically during the shelf-life storage from the initial 0,2 ms^{-2} to 0,05 ms^{-2} and 0,14 ms^{-2} to 0,05-0,06 ms^{-2} in case of Bosc kobak (Fig. 3 left) and Conference (not shown), respectively.

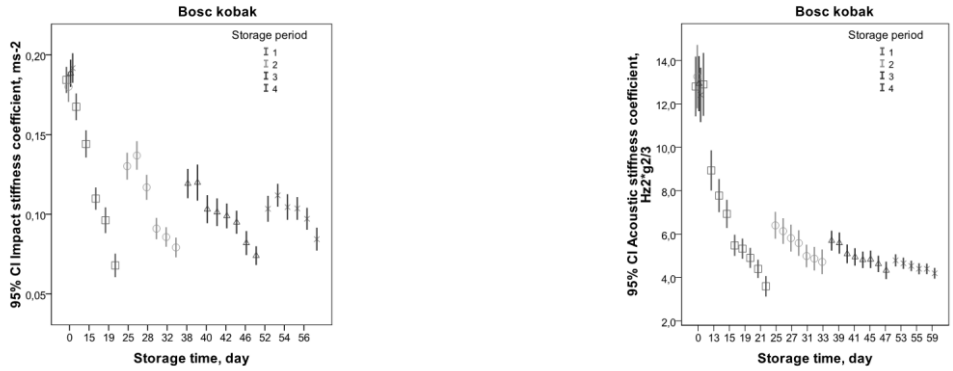

Figure 3: The changes of the impact and the acoustic stiffness coefficient vs. storage time (Bosc kobak variety)

It clearly represents the effect of the storage temperature on texture changes. In case of Bosc kobak variety, the acoustic stiffness' monotonous change can be observed (Fig 3. right) during the entire storage period, but in case of Conference, after the initial decrease the change of the acoustic stiffness coefficient was not significant (data not shown).

The total chlorophyll content related FWHM value of the samples increased during ripening, representing the decrease in chlorophyll content. In Fig. 4, the chlorophyll content decreased slowly at low temperature (the first measured value point of the storage period represents the cold storage) and at room temperature faster. The two different varieties behaved clearly differently, concerning the range of the Slope (-1.9 to -1.2 and -1.2 to -0.5, data not shown) and FWHM (19 to 27 and 30 to 46) values. Significant differences were found between the initial data and the data of the withdrawals in case of the Slope and FWHM values.

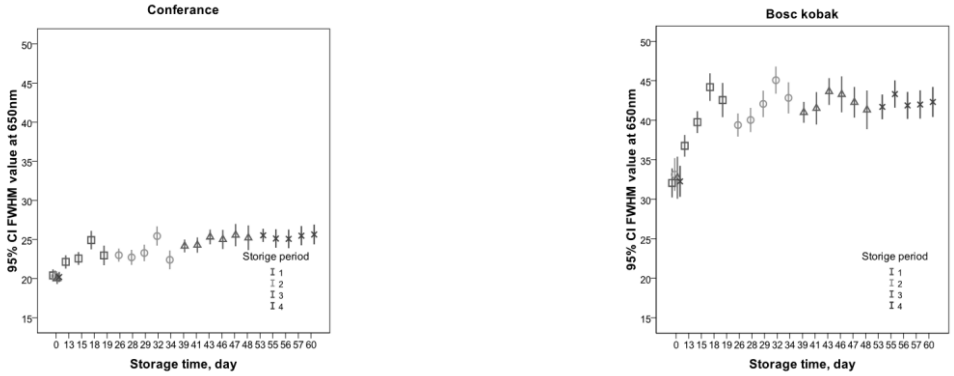

Figure 4: Change of FWHM backscattering imaging characteristic 650nm laser light sources vs. storage time

4. CONCLUSIONS AND SUGGESTIONS

All the applied non-destructive measuring methods were found to be suitable for the monitoring of the pear variety dependent postharvest quality changes during cold and subsequent shelf-life storage at simulated retail conditions, but with different kind of intensity.

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LIFE CYCLE ANALYSIS FOR ENVIRONMENTAL IMPACT OF LIQUID FOOD PACKAGING

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SUMMARY

It is a general tendency, that the development of food packaging systems and the food consumption go hand in hand. A striking example of this phenomenon is the drink-water consumption: the water from the tape has been replaced by mineral waters in polyethylene terephthalate (PET) bottles, the "loose milk" is diminished, and emerged new, high tech packaging solutions. These processes have considerably increased the environmental burden, caused by the food chain. To understand the complexity of environmental burden, new, computer-supported life cycle analysis databases and software have been developed all over the world. These tools serve mainly the assessment of environmental impacts of different packaging systems, but can serve as a suppliers of input for managerial decision makings, taking into consideration the market demand, regulatory framework and resources.

FOOD INDUSTRY AND THE INTERNET: INNOVATIVE METHOD FOR PRODUCT DEVELOPMENT

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SUMMARY

As a result of the fast innovation activity the so called „Many to many" communication channel has become one of the most important tools for the Hungarian food industry. The number of Internet users in Hungary was 6.5 million in 2011, that represents 65,4% of the total population. The Internet based public media provides a strong and active platform between food producers and consumers. The online platform gives up-to-date and precise information about food companies and food products to the consumers. The information flow is two-directional as food companies receive a precise overview on consumers, their habits and latent needs by using innovative market research methods.

The aim of this paper is to evaluate the media communication of some of the most important food companies and their products. We used data mining techniques to get more precise and the latest information about Hungarian food consumers. This survey supports more efficient marketing communication and strategies for the innovation of the food products.

1. INTRODUCTION

Beside the ordinary media, like newspapers, radio, the Internet is considered as a young mass communication channel. Compared to other media the development of the Internet is much more dynamic. The Internet users of the world population were 16 million (0.4 %) in 1995. Fifteen years later the number of users increased to 1,650 million (15.7%) in 2010. The latest statistics prove that the number of Internet users is 2,749 million which means 39 percent of the world population. The average increase in the number of Internet users is 5-10 percent yearly. (www.internetworldstats.com).

Parallel to international tendencies a change in the Hungarian media consumption can also be pointed out. According to the Hungarian market research company, GfK, in 2005 on average the Internet was used 7.9 hours a day by Hungarian adults that is the Hungarian Internet users' population (33% of the Hungarian population) used the Internet 23.8 hours a month in 2005. Five years later an average Internet user spent 22.9 hours on the Internet in 2010. Altogether 58 percent of the Hungarian population spent 39.5 hours with using the Internet. (Kántor, 2011). On the basis of the increase of Internet subscriptions half of the Hungarian population is available through the Internet. These significant statistics encourage business companies and food producers to make more efficient contact with their clients through the Internet. The Internet communication has become a vital source for companies. In the strong competitive environment the up-to-date and precise information means a comparative advantage for businesses. With the use of this information they can make a prompt survey on consumers' needs and their changing attitude ignoring the time consuming old, traditional methods.

The most important platforms for communication are social media. Social media is based on Internet 2.0 web application and enable users to create contents and use them. (Kaplan & Haenlein, 2012)

Nair (2011) defined social media as the collection of tools the elements of which are content and opinion sharing, the media and contacts and relations between business companies and Internet users. According to another definition social media is an online information source that is created by users to share information and to call attention to products, brands, services, personalities and topics. (Blackshaw & Nazzaro, 2004). In the

traditional media information is mainly produced by companies and is spread in one direction (one to many).

In the social media information are produced by users and they spread them. It is the many to many form of information flow. (Tariq & Wahid, 2011) This type of flow brings the democratization of information and knowledge (Evans, 2008). Contents are transformed to consumers' contents that has changed the mode of communication between companies and consumers. This process has changed companies' communication as they have become part of consumers' communication.

2. MATERIALS AND METHODS

The most popular social media websites are Facebook, IWIW, and MyVip in Hungary. Taking the registered and active users Facebook and IWIW have dominance on the Hungarian social media market. Facebook, the focus of our research, is the largest and the most popular social website in the world. Noeadays, with more than one billion users Facebook has become a kind of multimedia centre. (Wikipedia, 2013) According to the statistics of April 2013 the number of Facebook users is 3 millions. They represent approximately 30 percent of the Hungarian population. The proprtion of male users is 48 percent and that of female users is 52 percent.

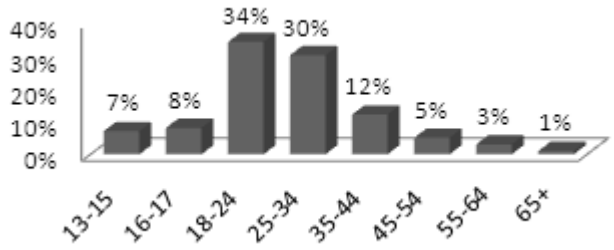

Figure 1: Ages of users on the Facebook in Hungary (Bereczki, 2011)

Considering the generation proportion of Facebook users, the vast majority of users is the age of 18-34, who represent two millions users from the above mentioned three million. (Figure 1) The older generations use the Internet in an insignificant number. The proportion of the 55 year-olds or more is only 4 percent. (Bereczki, 2011)

The websites users can express their opinion about a website, a company or a product with the possibility of using "Like" panel on websites. Through the analysis of their liking activities on the Facebook, this paper presents the research on the food consumers' preferences and needs. The research topic and the huge number of Facebook users enable a wide range of comparative analysis on the decision making and support to discover the differences between consumers' groups. The knowledge about consumers' attitudes and needs of different consumers' groups contribute to a more efficient way to position and plan of product developments. In contrast with the traditional surveys like questionnaires the Internet surveys provide prompt and real information about the target group.

We made the analysis with the Wisdom software and its version Professional that was developed by MicroStrategy. The Wisdom Professional basis on a Wisdom Network community that has 22.9 million users. The enrolment to this community is voluntary. For little advantages the members share their datasheets with their personal data like age, home address, educational level, website preferences. These data are collected in a database. The Wisdom software is a unique and efficient consumers' research method that can be applied to discover information about consumers using their Facebook database. The software offers a wide range of demographical data, interest fields, activities and motivations about website

users. It is efficient in segmentation of consumers' groups that can support a more efficient marketing and product development activities.

3. RESULTS

This paper presents the result of our research on Coca-Cola, one of the worldwide known brand. On the basis of the survey on Coca Cola it had a number of 67.1 million "Like" in June 2013. This figure shows the number of users' satisfactions with the Coca-Cola. To tighten the sample of our selected product we analysed the data of Coca Cola Light at international and domestic level.

In line with the international result the analysis highlighted that consumers who liked the product with reduced sugar content belong to the younger generations. Most of them live in towns and have higher educational level. The proportion of male consumers who like Coca Cola Light is 32 percent while females have a larger proportion with 68 percent. The Table 1 summarises the results of the analysis. According to the marital status singles have the largest share in the sample (45%). The engaged users are in the second place (33%) and married users represents 18 percent in the third place.

Table 1: Share of Coca-Cola Light likers in Hungary by marital status

Relationship Status	Single	45%
	In a Relationship	33%
	Married	18%
	Engaged	4%

Source: own calculation

The analysis of preferences and educational levels domestically, in line with the international results, shows that the users with college degrees like the Coca Cola Light product the best. They are followed by the high school students' number of liking. Users with university degrees has an ignorable share in the sample. (Table 2)

Table 2 : Share of Coca-Cola Light likers in Hungary by educational level

Education Level	High School	40%
	Collage	45%
	Graduate School	15%

Source: own calculation

We analysed the users by the average salary, as well. The less salary means the more liking of the Coca Cola consumption. This result in Hungary is in accordance with the international results.

Beside the socio-demographic analysis the website provides a possible to examine the users' liking of other food companies or food products and how they consistent in healthy food choice (i.e. sugar free products). Surprisingly the results did not justified the conusmers' consistency. The users' majority who like Coca Cola Light products like other food products like Túró Rudi (Sweet cottage cheese), Nutella, Monster, with a high rate of sugar content. The Table 3 shows that the half of the users who like Coca Cola Light expressed their liking in websites with a converse content to health products. This result differs from the international tendency. While the international results present a one-third share, the 48.9 percent of the Hungarian consumers are antinomic in liking activities. Affinity index shows the changes in the last years.

Table 3. The interets of Coca- Cola Light likers in Hungary

Page	Affinity	% of Segment
Túró Rudi (Sweet cottage cheese)	1518.4x	76.1
Coca Cola	13.8x	59.8
Nutella	34.2x	50.0
Cool to have Meki!	2627.6x	48.9
McDonald's	21.6x	47.8
Monster Energy	29.2x	46.7
Milka	2702.5x	43.5
McDonald's Hungary	8709.8x	43.5
Milka	381.0x	42.4
Red Bull	15.7x	41.7
Starbucks	6.8x	35.9
Sprite	43.4x	33.7
Ferrero Rocher	22.9x	31.5
Norbi Update	1749.4x	30.4

Source: own calculation

4. CONCLUSIONS AND DISCUSSION

The spread of the Internet mass communication tools enable to make direct survey on food consumers who visited a website and expressed their liking on a certain product, service or company. As consumers visited the website the product has made an impact on them. The analysis of liking users provides a very precise overview on consumers' behaviour, socio-demographic characteristics, membership of a certain type of group and their food consumption habits. The results of the analysis support to discover the latent consumer needs that help a more efficient development and marketing of food products. The application of the analytical software introduced in the paper started a few months ago in Hungary. The outcomes of the application have greatly contributed to company management and the target oriented product development.

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NUTRITION AND FOOD HABITS OF SCHOOL CHILDREN IN URBAN AND RURAL AREAS OF THE MUNICIPALITY OF TRAVNIK

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SUMMARY
Adolescence is an important period of forming and adopting proper eating habits that should be the basis of good health. The aim was to examine the nutritional status and dietary habits of children in urban and rural environments, and their attitudes and knowledge about the importance of healthy eating as well as physical activity through further growth and development. The study included 229 students from urban areas and 218 students from rural areas of both sexes, 11-14 years old. The measuring instruments that were used is a questionnaire (22 questions related to the anonymous views on dietary and lifestyle) and anthropometric measurements (body mass index). The research results of the total number of respondents to percetile curves showed that 160 (70%) of students from urban areas had a desirable body weight, and 149 (69%) of students from rural areas. Obesity in urban areas is higher 17 (8%),than in rural is 6 (3%) which is almost three times higher. Various workshops and introduction of teaching curriculum in schools on this subject over time should allow the raising of awareness in forming healthy eating habits from an early age.

1. INTRODUCTION

Nutrition depends bouth on the quantity and quality of food and the ability of the body to use energy , component and protective materials. (Simić B., 1998.). In assessing the health of the population, nutritional testing is one of the most important parts. Proper nutrition is proven by examining growth and development, the absence of clinical signs of deficit or surplus in nutriton, proper body functions and nutrient content of ingredients and protective supstances in the body. (Kristoforović – Ilić M., 2003.). Nutriton is affected by a number of causes: genetic, socio – economic, demographic, dietary habits, physical activity. It is particulary important to understand the nutritional status of children due to the growth and development, and to recognize individuals who deviate from established nutrition criteria for children of a certain age. . (Šelović A., Jureša V. 2001; 4:159-65). Normal BMI (Body Mass Index) occurs if the optimal intake of energy, component and protective materials, functions and structures of the body are normaly good and unchanged, and reserves of these materials are sufficient to maintain normal function and structure, even in the case of short – term and in sufficient nutrition. Therefore, this condition correspondes to complete saturation of the organism in terms of all the necessary materials. Inproper, one – sided and inadequate nutrition can cause warrious this orders and even the appearance of clear signs of diseases. The food we consume directly affects our well being, as well as health. (Simić B., 1998.). Malnutrition interferes with normal growth, development and limits the realisation of genetic potential. Overweight and obesity are risk factors for the development of a number of diseases, especially cardiovascular. Obesity is one of the five major risk factor for cadriovascular diseases in children. (Dumić M., Špehar A., Janjanin N. 2004;1:3-7). The aim of this paper was to examine the nutritonal status and dietary habits of children ih urban and rural areas as well as there attitudes and knowledge abouth the importance of healthy eating and physical activity through further growth and development.

2. MATERIALS AND METODS

The survey was conducted from March ti June 2013 in schools in the CBC (Central Bosnian Canton). The study enrolled schools in urban and rural areas. It was anonymous, with the voluntary consent of the participants. Data was used from a survay of 447 students aged

11 to 14. Out of these 229 students were from urban areas and 218 students from rural areas. The measuring instruments that were used is a questionnaire (22 questions related to the anonymous views on nutrition and lifestyle) and anthropometric measurements (BMI), and presents the results of an assessment of nutritional status of school children examined according to BMI for age related to gender. The questionnaire consists of four parts: general information (name, school, place of residence, grade, age and gender), dietary habits (number of meals, the frequency of consumption of cooked food, breakfast before school, choice of snacks, food choices at school during recess, and if they consume more water or juices), attitudes about nutrition (how often they eat fruits, vegetables, sweets, snacks, cereals, white or brown bread and how often they drink carbonated drinks) and lifestyle (physical activity, tobacco, alcohol). Data was statisticaly analysed. Based on anthropometric measurements (BMI) and age, we calculated percentile that gave us the assessment of the nutritional status of each child.

3. RESULTS

Results according to the precentile curves showed that 160 (70%) of students from urbanareas had a desirable body weight, and 149 (69%) students from rural areas. Obesity in urban areas is higher 17 (8%),than in rural is 6 (3%) which is almost three times higher, which is to be expected due to sedentary lifestyles and eating habits, as well as greater availability of unhealthy foods. (Table 1.)

Table1: Nutrition data in urban and rural areas according to percentile curves

Nutrition data	Urban areas	Rural areas
Obesity	17 (8%)	6 (3%)
Overweight	46 (20%)	52 (24%)
Normal weight	160 (70%)	149 (69%)
Malnutrition	3 (1%)	7 (3%)
Severe malnutrition	3 (1%)	2 (1%)

Regarding to the question of the number of meals per day in urban areas 3 (1%) students have one main course meal a day, in rural none 0 (0%). Two main course meals a day have 42 (18%) of children in urban areas and 72 (33%) in rural areas, while three main course meals have 184 (81%) in urban areas and 146 (67%) of rural children. When asked about the consumption of cereals (cornflakes) between urban and rural children results are as follows: 53 (24%) of students in rural areas consume cereals once a week, while in the urban areas only 6 (4%). The number of students that consume vegetables every day, several times at day, in rural areas is 49 (23%), while in urban areas 27 (12%). This can be explained by the fact that fruits and vegetables are more accessible to children in rural areas because of the way of life and their environment. As for the frequency of consumption of brown bread results show that 65 (39%) of students in rural areas have never consumed brown bread, and in urban areas 47 (21%). By analyzing the obtained data about the consumption of carbonated beverages it showed that in rural areas 37 (17%) of students consume once a day, every day, and in urban areas 21 (9%), which indicates the more developed awareness of children in urban areas of the harmful effects of consuming carbonated beverages. Such eating habits suggest the need for education of students, their parents, as well as subjects in schools involved in the preparation of school meals on the principles of proper nutrition and its impact on health.

The higher level of sports activities in children from rural areas has been confirmed in other studies. 197 of them (86%) in rural areas engage in physical activity, while 160 (73%) in urban areas. One of the reason that authors often quote that rural children prefer to play

team sports like football, basketball and handball, where it develops aerobic capacity significantly, while urban children tend to indoor sports or sports where aerobic component are expressed. Furthermore, the reason for lack of action and physical exercise in students from urban areas, may be the lack of sports facilities and adverse weather conditions and lack of knowledge of the importance of physical exercise. (Figure 1.)

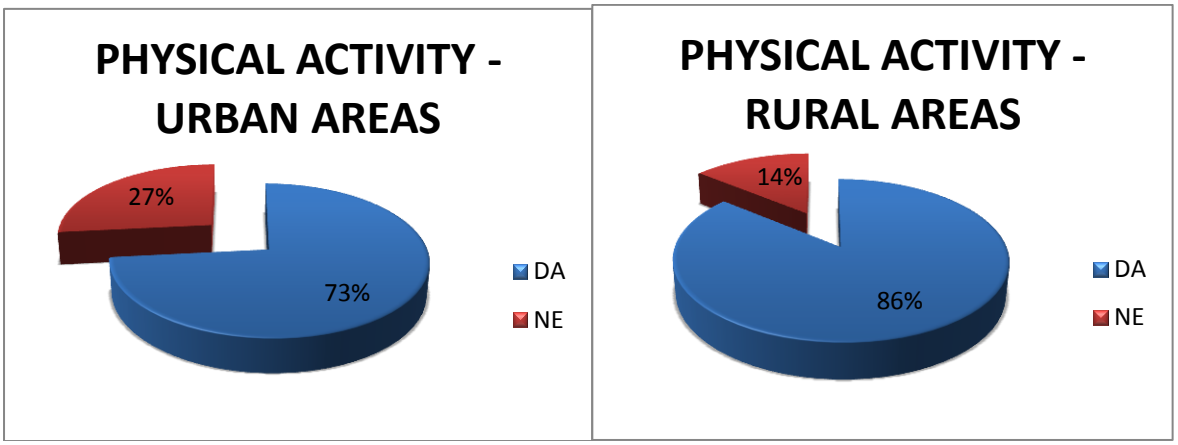

Figure 1: Physical activity in urban and rural areas

As for the frequency of consumption of cigarettes in urban areas gave the following data, 6 (3%) students smoke cigarettes, 54 (25%) in rural areas. When asked about drinking, 218 (95%)in urban areas do not consume alcohol, and 179 rural (82%), while 11 of them (5%) in urban areas consumed alcohol, and 39 (18%) in rural areas. Peer pressure, crisis, youth fears lead to drinking and creates a false sense of calm, output from various problems and encouragement. To prevent alcohol among young people it is necessary to offer a more rational and better organization of leisure time, more useful gathering, where young people need to find themselves and a sense of satisfaction.

Taking into account the statistics and findings of nutritional status and quality of nutrition of the population in Croatia during 2012 in Zagreb a sample from was reviewed children age 6 – 15 out of which 487 boys and 462 girls. Analysis of the results show that the (WHO) reference values 65.8 of the examined students normally nourished, 1.2% are underweight, while 32.6 % of the measured children are overweight, from 19.4% had increased body weight, 11.3% were obese, and 1.9% were extremely obese. Comparing eating habits of school children in Croatia, 38.9% of students consume fruit, while a number of students who eat vegetables every day is only 26.7%. (http://www.hzjz.hr/publikacije/00_2012_WEB.pdf)

Center for Disease Control Atlanta, World Health Organization and the Canadian Association of Public Health began research in the year 2000., Global Yuoth Tobacco Survey (GYTS), whose purpose is to monitor and review the problem of tobacco use among young people aged 13 – 15 on a global scale. Serbia participated in this project in 2003. Results showed that 54.7% of young people aged 13 – 15 have tried cigarettes, and one third of them by the age of ten. (Stojiljkovic et al., 2008). The data obtained suggest that the smoking starts early and requires aggressive implementation of health – educational programs for the lower grades which in addition to providing information about the dangers of tobacco would help develop certain skills and techniques that will enable them to resist offers of the others and peer pressure to begin consumption of tobacco.

4. CONCLUSION

Based on data obtained we can conclude that an unbalanced diet whether it is insufficient or overabundant, compared to the actual needs, leading to a negative or positive balance, to malnutrition or obesity, especially if it is disorderly and deficient in the structural and protective materials, has a negative effect on nutritional status, as well as the general state of health, and therefore has a negative effect on the ability to work. The fact is that obese children are candidates for obesity in adulthood. Because obesity is a proven risk factor for more massive chronic diseases, primarily those of cardiovascular and type 2 diabetes, it is important to discover on time the children that are more overweight or are already obese, and implement targeted programs of preventive measures and activities to promote a healthy lifestyle. With regard to the information shown on the nutritional status of children we can concluded that there are no problems with deviation from normal weight. Activities to improve the diet should focus on addressing nutritional deficits, particularly iron deficiency anemia, and the promotion of healthy eating and regular moderate physical activity from the earliest days, starting from kindergarten and then throughout their schooling. Various workshops and introduction of teaching curriculum in schools on this subject over time should allow the raising of awareness in forming healthy eating habits from an early age.

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ANALYSIS OF CONSUMER PREFERENCES RELATED TO FOOD ADDITIVES IN HUNGARY, SPAIN AND ROMANIA: RESULTS OF A CHOICE BASED CONJOINT ANALYSIS

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SUMMARY

In order to better understand the preferences related to foodstuffs containing food additives, a choice-based conjoint analysis was completed. The study was performed with the help of two "model foodstuffs": pre-packed sliced cheese and chips. For the creation of the conjoint cards three attributes were selected: "preservatives" (natural/artificial), "packaging gases" (contains/ does not contain) and "price" (average based on market data/+10%/+20%). Results were collected via Internet and data were analysed with the Conjoint module from the XLSTAT statistical software. In the analysed countries "natural preservatives" highly positively contributed to the choosing of both cheese and chips, while the too high price (+20%) had negative impact on it. The most important factor was the "preservatives" in both cases. No relevant differences were observed between the two selected food products.

1. INTRODUCTION

Consumers expect from a foodstuff to be convenient, tasty and pleasant to eat among other aspects. In order to fulfil these consumer demands, the food industry uses different food additives. However more and more people try to avoid foodstuffs containing food additives and try to consume products supposed to be safer, healthier and natural products. To analyse consumer preferences related to food additives three countries were selected: Hungary, Spain and Romania. According to the survey of the Eurobarometer (2010) level of worry against food additives was different in these countries. In Hungary the rate of concern about food additives was high (81%), while in Romania (74%) and Spain (54%) lower. The aim of our study was to analyse and compare the preference of foodstuffs from the point of view of food additive content in three European countries.

2. MATERIALS AND METHODS

2.1. Conjoint analysis design

According to the authors' previous survey Hungarian respondents judged "preservatives" to be more hazardous than Spanish people, while in case of "packaging gases" the opposite tendency was observed. Furthermore, unambiguous consumer demand was found for foodstuffs free of food additives even at a higher price in each country. Thus for the conjoint study three factors were chosen: "preservatives" (artificial/natural), "packaging gases" (contains/does not contain) and "price" (average+10%/average+20%). Average prices were determined on the basis of market data. Conjoint cards of two "model foodstuffs" were created and used to simulate a shopping situation. One foodstuff expected high additive content (chips) and one expected low additive content (pre-packed sliced cheese) (Tarnavölgyi, 2009; Szűcs & Bánáti, 2010). For the choice based conjoint study six cards were chosen and one more to illustrate the "standard" foodstuff (contains artificial

preservatives and packaging gases on average price). In the choice sets the cards were compared to the "standard" foodstuffs' card.

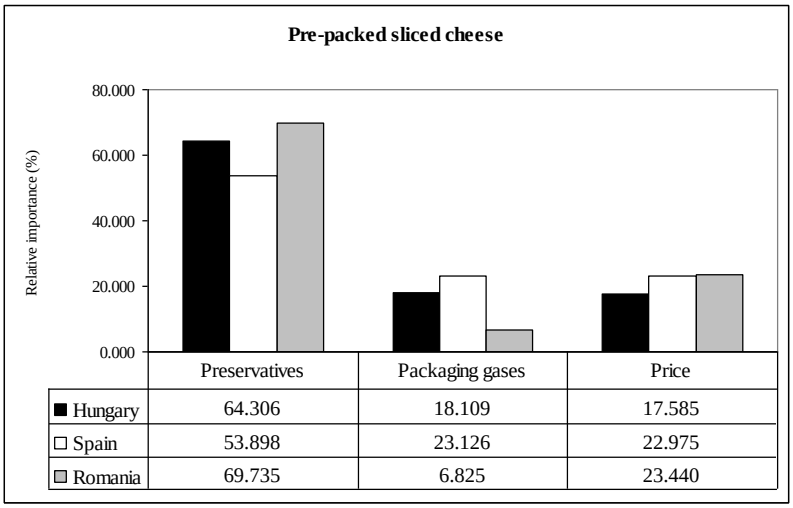
2.2. Conjoint analysis data collection and statistical analysis

Results were collected via Internet. A total of 214/210 (pre-packed sliced cheese/chips) valid questionnaires were kept in Hungary, 154/157 in Spain and 130/101 in Romania. Estimation of the utility values and the relative importance that participants gave to the selected attributes during the foodstuff selection was performed with the Conjoint module from the XLSTAT statistical software.

3. RESULTS

3.1. Relative importance and utility values of pre-packed sliced cheese

Value of the relative importance refers to the importance of an attribute within the choice process. In all cases "preservatives" was the most important factor in the choice decision. "Packaging gases" was more important for the Spanish participants, while "price" for the Romanian ones. For the Hungarian and Spanish participants "packaging gases" and "price" had pretty much the same importance when buying a pre-packed cheese. However for the Hungarian respondents "preservatives" was more than three times important factor as "packaging gases" and "price", while for Spanish ones "preservatives" was twice as important attribute. According to the Romanian respondents "packaging gases" had a small importance in the shopping decision of pre-packed sliced cheese. Nevertheless "preservatives" was ten times important as "packaging gases", and thrice important as "price" (Figure 1).



	Preservatives	Packaging gases	Price
Hungary	64.306	18.109	17.585
Spain	53.898	23.126	22.975
Romania	69.735	6.825	23.440

Figure 1: Relative importance of the attributes for pre-packed sliced cheese choice separated by country

The utility values reflect the relative contribution of the attributes to the consumers' choice of the choice set. Positive values mean positive contributions to the choice situation, while negative values reflect negative contributions. "Natural preservatives" had significantly positive impact in the pre-packed sliced cheese choice, while "artificial" had significantly negative in all countries. In Spain the presence of the "packaging gases" had significantly positive impact on the preference of pre-packed cheese, however the too high "price" had significantly negative impact. Price 10% above the average had positive influence on pre-packed cheese choice in Hungary and Spain, while 20% above had negative in the three analysed countries (Table 1).

Table 1: Utility values and standard error for the contribution of the factors to consumer choice of pre-packed sliced cheese separated by country

Factor	Level	Hungary (a)		Spain (b)		Romania (c)		Sig. $p \leq 0.05^{\square}$
		Utility	SE	Utility	SE	Utility	SE	
Preservative	**natural**	1.390$^{\bullet}$	0.147	1.482^{x}	0.128	1.712$^{\lozenge}$	0.164	a-b,c; b-c
	artificial	-1.390$^{\bullet}$	0.147	-1.482^{x}	0.128	-1.712$^{\lozenge}$	0.164	a-b,c; b-c
Packaging gases	**presence**	0.392	0.145	0.636^{x}	0.113	0.168	0.176	a-b,c; b-c
	absence	-0.392	0.145	-0.636^{x}	0.113	-0.168	0.176	a-b,c; b-c
Price	**average**	-0.152	0.250	0.583	0.208	0.590	0.278	a-b, c
	+10%	0.456	0.185	0.098	0.140	-0.030	0.192	a-b,c; b-c
	+20%	-0.304	0.169	-0.680^{x}	0.148	-0.560	0.202	a-b,c; b-c

$^{\bullet}$ Significant difference in Hungary according to Chi-square test
^{x} Significant difference in Spain according to Chi-square test
$^{\lozenge}$ Significant difference in Romania according to Chi-square test
$^{\square}$ Significant difference between the countries

3.2. Relative importance and utility values of chips

"Preservatives" had dominant importance in the choice decisions of the participants. "Packaging gases" was the more important factor for the Romanian respondents, while "price" for the Hungarian ones. In Hungary "packaging gases" was the less important attribute. "Preservatives" was twice important factor as "price" and thirteen times important as "packaging gases" in the choice decision of Hungarian participants. For Spanish respondents "preservatives" was more than twice important as the other two attributes. "Price" was the less important attribute for the Romanian participants. "Preservatives" was nearly twice important as "packaging gases" and six times important as "price" in Romania (Figure 2).

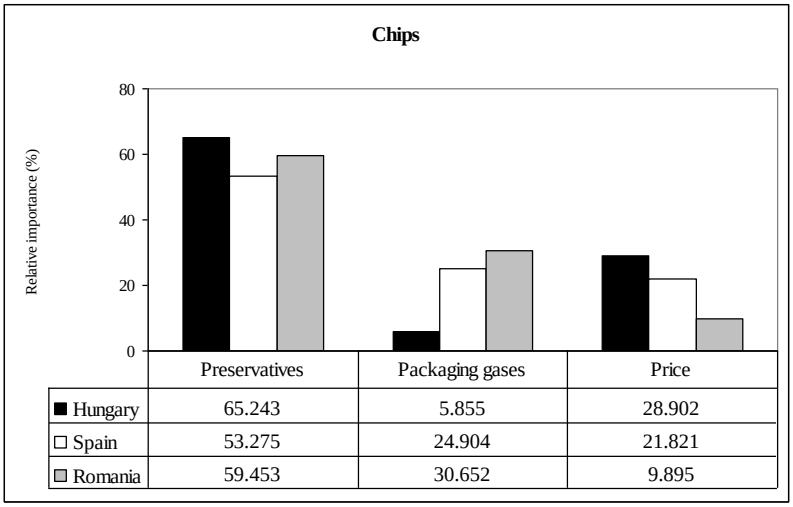


Figure 2: Relative importance of the attributes for pre-packed sliced cheese choice separated by country

As it was found in the case of pre-packed cheese, "natural preservatives" had significantly positive impact on the choice decision of chips, and "artificial preservatives" had significantly negative impact. Presence of "packaging gases" had highly positive effect in the selection of chips in Spain and Romania. However in Hungary had negative impact on the decision. "Price" 10% above the average had positive impact in the three countries, while "price" 20% above the average had significantly negative impact on the choice in Hungary and Spain (Table 2).

Table 2: Utility values and standard error for the contribution of the factors to consumer choice of chips separated by country

Factor	Level	Hungary (a)		Spain (b)		Romania (c)		Sig. $p \leq 0.05^{\square}$
		Utility	SE	Utility	SE	Utility	SE	
Preservative	**natural**	1.737$^{\bullet}$	0.143	1.498^{x}	0.128	1.353$^{\lozenge}$	0.160	a-b,c; b-c
	artificial	-1.737$^{\bullet}$	0.143	-1.498^{x}	0.128	-1.353$^{\lozenge}$	0.160	a-b,c; b-c
Packaging gases	**presence**	-0.156	0.146	0.700^{x}	0.115	0.698	0.202	a-b, c
	absence	0.156	0.146	-0.700^{x}	0.115	-0.698	0.202	a-b, c
Price	**average**	0.586	0.226	0.533^{x}	0.209	-0.124	0.312	a-b,c; b-c
	+10%	0.367	0.159	0.161	0.142	0.287	0.208	a-b,c; b-c
	+20%	-0.953$^{\bullet}$	0.170	-0.694^{x}	0.147	-0.163	0.219	a-b,c; b-c

$^{\bullet}$ Significant difference in Hungary according to Chi-square test
^{x} Significant difference in Spain according to Chi-square test
$^{\lozenge}$ Significant difference in Romania according to Chi-square test
$^{\square}$ Significant difference between the countries

4. CONCLUSIONS

On the basis of the choice based conjoint analysis with the involvement of three countries' participants it can be concluded that "natural preservative" had dominant impact in both foodstuffs' preference. "Presence of packaging gases" had highly positive effect on the selection of cheese and chips in Spain. For Romanian respondents the "presence of packaging gases" was also a positive attribute, mainly in the selection of chips. Hungarian participants' selection decision was negatively influenced by the "presence of packaging gases" in case of chips, and weakly positively in case of pre-packed cheese. Regarding the "price" it seemed that 10% extra money resulted positive decision, however 20% proved to be too high. "Preservative" had an outstanding importance in the both products' choice decision in the analysed countries. Consumer judgement of the foodstuffs from the point of view of food additives had influence on the choice decision. Results point out that substitution of "artificial" preservatives with "natural" ones can influence the analysed countries consumers' food choice decisions favourably. During the new products' launching it is important to take into consideration the "price", it could also result rejection. Furthermore it is important to note that no relevant differences were observed between the two selected food products.

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SECTION 4: Food safety and quality control; Nutrition


IN VIVO URINARY METABOLITES OF CHLOROGENIC ACID COMING FROM COFFEE OR APRICOT JUICE CONSUMPTION

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SUMMARY
Information on the bioavailability and metabolic fate of dietary phenolics is a key part of the equation in understanding their potential effects on human health. The metabolic fate of 5-caffeoyl-quinic acid, which is the major chlorogenic acid in coffee and also in apricot juice has been investigated in a human study. Analyzing urinary metabolites is a good approach to evaluate polyphenol absorption and metabolism that takes place in the human body. The aim of this work is to use HPLC-DAD-ESI-qTOFMS to identify polyphenol compounds excreted in urine after the acute consumption of coffee or apricot juice by healthy human subjects and provide discussion on the possible food matrix effects on the bioavailability and metabolism of chlorogenic acid.

1. Introduction

An impressive list of health-beneficial effects has been associated to polyphenol consumption, however their specific mechanisms of action are still not fully understood at the molecular level. In order to be able to assess the true mechanisms of action of these compounds when we consume them as a part of plant-based diet, bioavailability and subsequent metabolism has to be taken into account (Holst and Williamson, 2008, Del Rio et al., 2013, Crozier et al., 2010). It is now clear that following ingestion, dietary flavonoids including chlorogenic acids appear in the circulatory system not as the parent compounds but in the forms of metabolites. In metabolic studies relating to polyphenols, compounds appearing in the bloodstream or excreted in urine after absorption from the gastrointestinal tract following consumption of a polyphenol-rich food are often referred to as bioavailable or *in vivo* metabolites. In most of the cases it can be realistically hypothesized that instead of the intact compounds, *in vivo* metabolites play inevitable role in the experienced (beneficial) biological effects of polyphenols. Chlorogenic acids, a group of compounds comprising hydroxycinnamates, such as caffeic acid, ferulic acid and p-coumaric acid, linked to quinic acid to form a range of conjugated structures known as caffeoylquinic acids (CQA), feruloylquinic acids (FQA) and p-coumaroylquinic acids (p-CoQA) (Stalmach et al., 2010). Coffee is the most well-known source of chlorogenic acids however, apricot fruits, an important fruit in Hungary, are also considered as a rich source of chlorogenic acids. In this study therefore, the characteristics of chlorogenic acid metabolism consumed in different food matrices such as coffee drink and apricot nectar is investigated.

2. MATERIALS AND METHODS

Commercially available instant coffee (Nestlé Green Blend) and apricot nectar (Rauch) containing minimum 40% fruit content from apricot purée were used in the study. Coffee beverage made of 3.4 g instant coffee in 200 ml water or 500 ml apricot nectar were fed to healthy volunteers after a 36 h low polyphenol diet, at 8 am on the day of the trial. All urine excreted over 24 h was collected over the following periods: 0-2 h, 2-5 h, 5-8 h and 8-24 h. Excreted volumes were recorded and aliquots of samples were subjected to analysis. Food containing low level of polyphenols and water was consumed during the day. Urine samples were centrifuged 16070*g* for 3 min at 4°C, and diluted in 1:1 with a solution containing sinapinic acid as surrogate standard, 20% MeOH and 0.2% formic acid (HCOOH). Apricot

nectar was extracted with 60/39/1 MeOH/water/HCOOH (v/v) solution for 30 min in an ultrasonic bath. Diluted apricot extract and coffee beverage were centrifuged and the supernatants were filtered through a 0.2-μm PTFE filter before HPLC-DAD-ESI-qTOFMS analysis. Chromatographic separation was carried out using a 150 x 4.6 mm column having 2.6 μm pore-sized, core-shell structured phenyl-hexyl stationary phase (Phenomenex Kinetex). Acetronitrile (ACN) and high purity water, both containing 0.1 % HCOOH were used as mobile phases. Mass spectrometric measurements were carried out with triple quadrupole and qTOFMS systems both equipped with ESI ion sources.

3. RESULTS

High-resolution, accurate-mass TOFMS technique along with UV detection was used for screening and identifying chlorogenic acid compounds in coffee beverage, apricot nectar and their metabolites in urine samples. The applied MS protocol (Abrankó et al., 2012) has been designed to be suitable for detecting and tentatively identifying a wide variety of suspected compounds i.e., there is no need to predefine the compounds of interest prior to analysis. The on-line coupled diode array UV detector provided supplementary spectral data for the tentative identification. First, the food samples (coffee beverage and apricot nectar) were screened in order to reveal the formes of quinic acid esters (chlorogenic acids) present in the samples. In the coffee beverage eight major forms, namely two caffeoyl-quinic acids (3-CQA, 5-CQA), three feruoyl-quinic acids (most probably 3-FQA, 4-FQA, 5-FQA) and three di-caffeoyl-quinic acids isomers were identified. In the apricot nectar, 3-CQA and 5-CQA were identified as major chlorogenic acids. It should be noted that each compound given above as well as thorough this work, was tentatively identified based on their accurate mass MS data including fragmentation and isotopic information. Since chlorogenic acids have absorption maxima around 330 nm, UV chromatograms recorded at this wavelength provide a demonstrative overview of the identified compounds (Fig. 1)

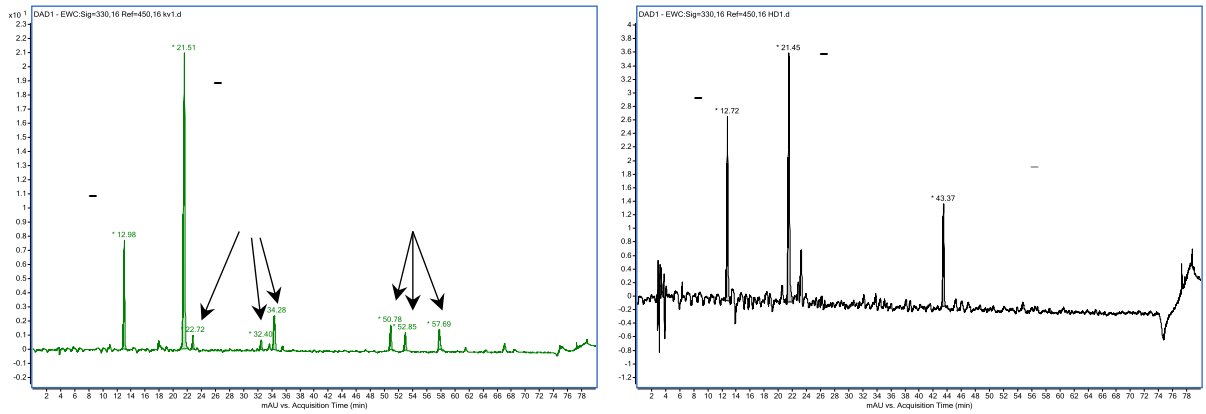

Figure 1: Detected and identified major chlorogenic acids in coffee beverage (a) and in apricot nectar (b). Chromatograms are recorded at 330 nm. The predominant (non-chlorogenic acid) peak in apricot nectar is quercetin rutinoside (rutin).

Reference standards were available only for 3-CQA and 5-CQA, therefore only these compounds could be quantified in the samples using an HPLC-ESI-MS/MS (triple quadrupole) system and UV detection. Accordingly, the studied coffee beverage and apricot nectar contained caffeoyl-quinic acids in total at 3.54 and 0.27 mM concentration respectively. The measured very low amounts of 3-CQA and 5-CQA in urine samples covered approximately the 1-2 % of the consumed amount. Feruoyl-quinic acids (most probably 3-FQA, 4-FQA and 5-FQA), which were present in the coffee drink were also detected in the urine samples excreted after coffee consumption. Interestingly, two additional feruoyl-quinic

acids were also observed in these samples at 26.53 and 37.20 min retention times (see Fig. 2). These compounds are supposed to be isoferuoyl-quinic acids formed as a result of enzymatic methylation of 3-CQA and 5-CQA during metabolism.

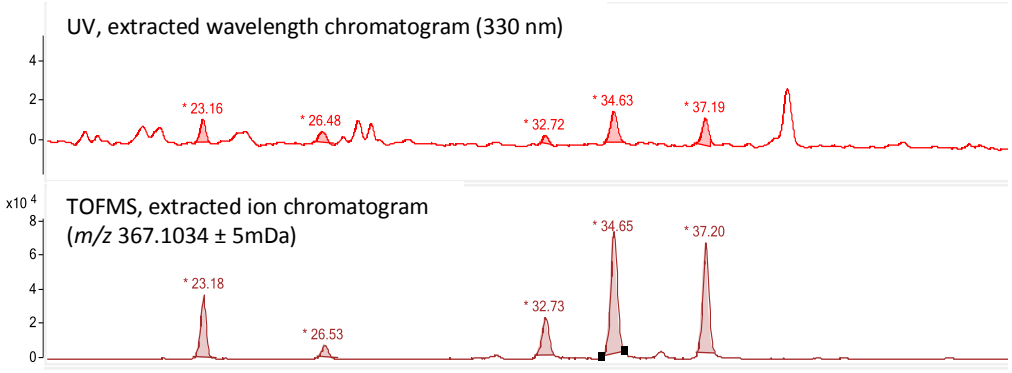

Figure. 2: Feruoyl-quinic acids (FQA) in urine samples after coffee consumption. Peaks at 23.2, 32.7 and 34.6 min are most probably 3-FQA, 4-FQA and 5-FQA, which are also present in the studied coffee beverage. The peaks at 26.5 and 37.2 min are also isomeric forms of FQA, most probably 3-isoFQA and 5-iso-FQA.

Additional urinary metabolites of chlorogenic acids were also observed. Sulfated conjugates of both caffeic and ferulic acid were identified, while only ferulic acid glucuronides could be detected. Unconjugated caffeic and ferulic acids could not be identified. The recently identified and published gut microbiota mediated metabolite, feruoylglycine was also detected in urine samples (Stalmach et al., 2010).

4. CONCLUSION

It has been demonstrated that only a minor portion of the orally consumed 3-caffeoyl-quinic acid (3-CQA) and 5-caffeoyl-quinic acid (5-CQA), which are the major chlorogenic acids in coffee and apricot, are absorbed in their intact forms in the gastrointestinal system. On the contrary, chlorogenic acids go through extensive metabolism resulting in methylated, sulfated and glucuronidated metabolites. The principles controlling absorption and metabolism in the small intestine are now broadly understood for some polyphenols, therefore it is hypothesized also in the case of chlorogenic acids that the small intestine is the most probable site for cleavage of quinic acid from the CQAs and FQAs and the release of caffeic acid and ferulic acid. Analogously to the metabolic fate of the most studied flavonols, cleaved ferulic and caffeic acids are sulfated through the respective action of sulfotransferases (SULT) in the epithelial cells of the small intestine before entering the bloodstream. In accordance with other studies it also has been demonstrated that a portion of FQAs is absorbed and thus these compounds appear in the urine as intact forms (Stalmach et al., 2009, Stalmach et al., 2010). However, our results indicate that some CQAs are not cleaved to caffeic acid and quinic acid before absorption. These compounds appear in urine samples as methylated CQAs, which are equal to isoferuoyl-quinic acids. Since all the three possible isomers of feruoyl-quinic acids (3-FQA, 4-FQA and 5-FQA) were equally present in the coffee beverage and in urine samples, the observed additional two FQA isomers might be isoferuoyl-quinic acids (isoFQAs) i.e., methylated CQAs. The observed compounds along with the indication of the supposed metabolic pathways are outlined in Fig.3.

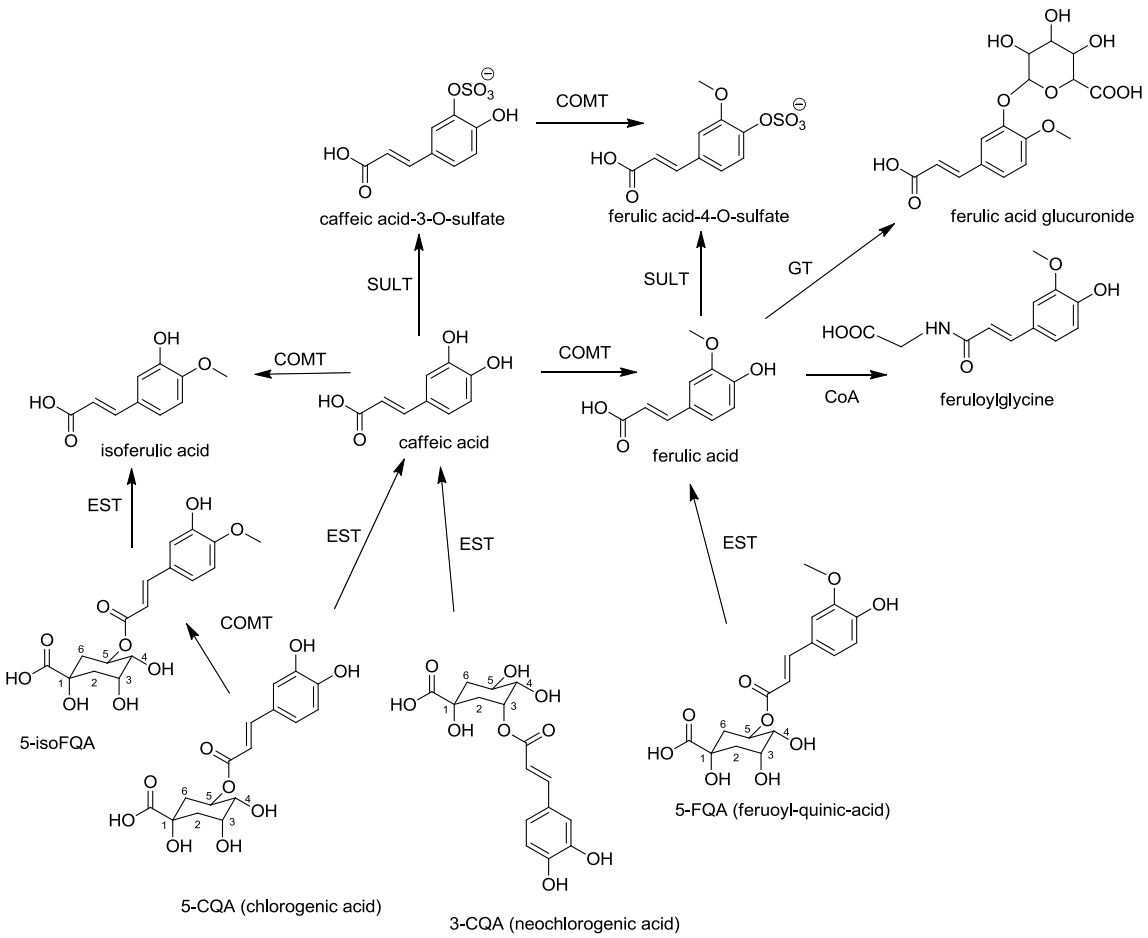

Fig. 3 Studied chlorogenic acids, their metabolites and possible metabolic pathways. COMT: catechol-O-methyltranferase, EST: esterase, UDP-glucuronosyltransferase, SULT: sulfotransferase

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INHIBITION OF *LISTERIA MONOCYTOGENES* BY POTENTIAL ENDOPHITIC BACTERIA ISOLATED FROM *CAPSICUM ANNUUM* VAR. *GROSSUM* CULTIVARS

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SUMMARY

Endophytic microorganisms live inside plant tissues without causing diseases and might have numerous positive effects on the host plants. Endophytes can promote the growth and yield of the plant, help to remove contaminants from the tissues, and can suppress growth of pathogens, however some enteric human pathogenic bacteria have also been isolated as endophytes. The aims of our study were the characterisation and identification of potential endophytic coliform bacteria isolated from different cultivars of sweet peppers (Capsicum annuum var. grossum) using a selective (VRBL) agar medium, and determination of antagonistic interactions between these potential endophytes and Listeria monocytogenes. The bacterial isolates showed heterogeneity based on their phenotypic and genotypic properties. Results of identification by molecular biological methods also confirmed the presence of different genera/species. Testing the antagonistic effect of the potential endophytic bacteria one isolate belonging to the dominant Pseudomonas genus showed significant inhibitory effect on Listeria monocytogenes.

1. INTRODUCTION

Food-borne pathogenic bacteria have notable significance in the food industry as they can contribute to economical losses and - what is more important - they can cause severe food-borne diseases. *Listeria monocytogenes* is one of the most dangerous pathogenic bacteria causing relatively rare but serious illnesses with high morbidity, hospitalisation and mortality rate. In 2011 the numbers of cases caused by *L. monocytogenes* in Hungary and in the European Union were 11 and 1476, respectively. Despite these relatively low numbers of infections mediated by *L. monocytogenes* this food safety problem is considered significant (EFSA, 2013).

Elimination of pathogenic bacteria from food and raw materials can be achieved by different conventional processing technologies (like heat treatment or application of preservatives), however demand for minimally processed food products has been increased in the last few years. Ready-to-eat salads do not require additional processing (or preservation) steps and are usually stored under refrigerated condition (at 4-5 ºC) which makes the presence of *L. monocytogenes* of particular concern.

Effective protection of foods or raw materials against pathogenic bacteria can be promoted by the application of antagonistic bacteria that are able to inhibit the growth of these harmful microorganisms. Endophytic microorganisms live inside the plant tissues without causing symptoms of diseases (Zinniel et al., 2002) and have positive effects on the plants. Endosymbiotic bacteria of plants can enhance the nutrient uptake by the hosts and protect them from pests and pathogens, thus they could contribute to the increased tolerance of the host plants against biotic and abiotic stresses (Clay & Schardl, 2002). Nevertheless, it was observed that pathogenic bacteria can internalise plant tissues and survive within the plant cells as endophytes (Berg et al., 2005; Ansingkar & Kulkarni, 2010).

The aims of our study were the characterisation and identification of potential endophytic coliform bacteria isolated from different cultivars of sweet peppers using VRBL agar medium, and determination of antagonistic interactions between the potential endophytes and *Listeria monocytogenes*.

2. MATERIALS AND METHODS

2.1. Isolation of potential endophytic bacteria from sweet pepper cultivars

Bacteria were isolated from different tissues – primary roots, stem, leaves and fruits – of hydro- and soil-cultures of *Capsicum annuum* var. *grossum* (sweet pepper) KPA and Ho cultivars using coliform selective violet red bile lactose (VRBL) agar.

2.2. Phenotypic characterisation of the isolates

The isolates were subjected to different phenotypic trials. Cell morphology was tested by light microscopy, while selected agar media (WL, Chromocult coliform, Pseudomonas P and Pseudomonas F) were applied for macro-morphological analyses. Oxidase-, catalase-, KOH- and OF-tests were used as biochemical and physiological assays.

2.3. Molecular biological analyses

Molecular biological analyses were used for characterisation and identification of the isolates. Rep-PCR using the M13 mini-satellite primer and amplified rDNA restriction analysis (ARDRA) using two different restriction endonucleases (*Msp*I/*Hae*III) were applied for typing purposes. *Pseudomonas* isolates were detected by a genus-specific PCR method and identified by direct sequencing of the *rpoB* gene. The non-*Pseudomonas* isolates were identified by sequencing the 16S rDNA PCR products and aligning these sequences with those deposited in the GenBank.

2.4. Testing of the interaction between *Listeria monocytogenes* and the bacterial isolates

Antagonistic effect of the isolated bacteria on *Listeria monocytogenes* CCM 4699 was determined by interaction studies using co-culturing and testing the inhibitory effect of the cell free supernatants.

3. RESULTS AND DISCUSSION

3.1. Results of phenotypic and genotypic analyses

Altogether 43 bacteria were isolated from primary roots, stem, leaf and fruit tissues, and grouped by STATISTICA 10 software based on phenotypic properties. The isolates showed heterogeneity, but phenotypically identical isolates could also be found between bacteria isolated from different parts of the plants. Using the M13 mini-satellite primer for rep-PCR the isolates were clustered into clonally related groups, in which – in contrary to the phenotypic alignment – only isolates from the same tissues and culturing conditions showed clonal identity. The results of restriction analysis of the 16S rDNA amplicons using two restriction endonucleases showed that this technique had lower discrimination ability compared to rep-PCR, however proved to be suitable for differentiation at genus level. A genus-specific PCR was used for the detection of *Pseudomonas* isolates. Amplicons were generated in case of 15 bacterial isolates, which indicated that approximately 1/3 of the isolates belonged to the *Pseudomonas* genus. Identification of these *Pseudomonas* isolates was done by sequencing the *rpoB* gene, while in case of twenty eight non-*Pseudomonas* bacteria the 16S rRNA gene was used as the target in sequence analysis. Results showed that

the isolated bacteria represented species of *Achromobacter*, *Agrobacterium*, *Comamonas*, *Delftia*, *Enterobacter*, *Erwinia*, *Leclercia*, *Mesorhizobium*, *Pantoea*, *Pseudomonas*, *Rhizobium* and *Serratia* genera, which refers to the presence of a diverse bacterial biota in/on the sweet pepper plants.

3.2. Determination of the antagonistic effect of the isolates on *Listeria monocytogenes*

Testing the antagonistic effect of the isolates on *Listeria monocytogenes* CCM 4699 by co-culturing inhibition was observed in case of seven isolates. The most significant inhibitory effect was attributed to one of the *Pseudomonas* isolates, which performed the best growth inhibition at 25°C. Growth curve analysis of *L. monocytogenes* CCM 4699 in the presence of cell-free supernatant of this *Pseudomonas* isolate at 25ºC showed that extracellular substance(s) even in a relatively low proportion could inhibit the growth of the pathogen, which indicated the potential biocontrol activity of this isolate. When supernatant was generated by cultivating the *Pseudomonas* strain at 30ºC the inhibitory effect could only be observed in case of higher supernatant proportion, while applying the supernatant at lower proportion stimulation of the pathogen was noticed.

4. CONCLUSIONS

Sweet pepper plants cultivated under different conditions harbour diverse bacterium populations as it was determined by molecular typing and identification of the isolates originated from VRBL coliform selective agar plates. Moreover, potential antagonists of food-borne human pathogenic bacteria among these isolates can also be present.

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ISOLATION OF BACTERIAL ENDOPHYTES AND ASSESSMENT OF THEIR BIODIVERSITY IN DIFFERENT *CAPSICUM ANNUUM* VAR. *GROSSUM* CULTIVARS

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SUMMARY

Endophytic bacteria known to reside in plant tissues and it has been proven that they might have important roles in plant vitality, seed germination ability and they may confer resistance to abiotic and biotic stress factors. Bacterial endophytes may have positive influence on the plants' physiology by producing phytohormons, and they might protect the host against plant pathogens through biofilm formation and antibiotic production.
Associated endophytic bacteria were isolated from different Capsicum annuum var. grossum cultivars grown in soil- and hydro-cultures, and identified by combination of traditional culture-dependent and molecular techniques. The potential endophytic nature of these bacteria was tested by using seed germination test.

1. INTRODUCTION

It has been proven that endophytic bacteria which are known to reside in plant tissues might have numerous beneficial effects on plant physiology like improved resistance against abiotic and biotic stress factors, production of phytohormons and antibiotics as well as protection against plant and human pathogens (Ryan et al., 2008).

On the other hand, it has been recently shown that the interaction between endophytic and human pathogenic bacteria could result prevention or - in some cases - even promotion of the penetration of pathogenic bacteria into the plant tissues (Teplitski et al., 2009).

Our aim was the isolation and identification of the associated endophytic bacterial communities from different *Capsicum annuum* var. *grossum* (sweet pepper) cultivars grown in soil- and hydro-cultures by combination of traditional culture-dependent and molecular techniques. Additionally, we aimed to determine the potential endophytic nature of these bacteria by using seed germination test.

2. MATERIALS AND METHODS

2.1. Isolation of potential endophytic bacteria

Samples were taken from different cultivars of *Capsicum annuum* var. *grossum* (from the seeds, primary and secondary roots, stem, leaves and fruits) grown under soil- and hydro-culture conditions. Bacterial isolates were cultivated by using TGY (tryptone-glucose-yeast extract) broth/agar, WL (Wallerstein) differential chromogenic agar and VRBL (Violet Red Bile with Lactose) coliform selective agar.

2.2. Phenotypic analyses

The bacterial isolates were tested for cell and colony morphology, Gram property, biochemical characteristics (catalase and oxidase tests) and spore-forming ability. Grouping of the isolates based on phenotypic characteristics was performed by STATISTICA 10 software.

2.3. Molecular typing

Molecular typing of the selected isolates was done by using RAPD-PCR method. Molecular fingerprints were clustered by GelCompar II software (Applied Maths NV, Belgium).

2.5. Identification of the isolated bacteria

One representative of the isolates from each RAPD-PCR cluster has been selected for molecular identification by sequencing the generated PCR products. Isolates belonging to the *Pseudomonas* genus were first detected by a genus-specific PCR reaction and the positive strains were identified at species level by sequencing the *rpoB* gene amplicons. Sequencing of the 16S rDNA PCR products was used for the identification of non-*Pseudomonas* isolates.

2.4. Seed germination test

Surface disinfected *Capsicum annuum* var. *grossum* seeds were gently shaken in bacterial suspensions for six hours followed by incubation in rolls of germination paper at 25 ^{o}C. Germination of seeds was evaluated visually after 7 and 10 days incubation.

3. RESULTS

A total of 171 bacteria were investigated, which originated from different parts of the plants and fruits.

The WL agar has been found to be a suitable culture medium to separate the isolates according to their macro-morphological properties.

Based on the obtained phenotypic characteristics and using the STATISTICA 10 software, it was possible to cluster the isolates into different phenotypic groups. Representative isolates from each phenotypic group have been selected for further molecular analysis.

It can be concluded that the isolates were mostly heterogenic in phenotypes, but there were some phenotypically identical isolates as well.

It was observed that clonally identical bacteria were isolated from the peppers grown under the same conditions (i.e. hydro- or soil cultures), and in most cases they originated from the same plant tissues.

Based on the results of molecular identification the isolates belonged to *Acidovorax, Agrobacterium, Brevibacillus, Cupriavidus, Delftia, Enterobacter, Leclercia, Leifsonia, Deinococcus, Bacillus, Microbacterium, Curtobacterium, Staphylococcus, Sphingobium, Paenibacillus, Pseudomonas, Rhizobium, Rothia, Stenotrophomonas* and *Xanthomonas* genera. Majority of the Gram-negative isolates were identified as *Pseudomonas* spp. and among the the others bacteria belonging to the *Paenibacillus* and *Stenotrophomonas* genera could also be detected in significant numbers.

It is worthwhile to mention that molecular identification based on the 16S rDNA and *rpoB* sequences resulted in more than one species or even genera for one isolate in many cases. This phenomenon may refer to the fact that these isolates are not yet properly identified, and investigation of further sequences has to be involved.

Majority of the isolates derived from the root tissues and just a smaller proportion originated from the above-ground plant tissues (leaves, stems and fruits). From the root tissues of the hydroculture-grown plants mostly Gram-negative bacteria belonging to the genera of *Pseudomonas* and *Stenotrophomonas* could be isolated, while in case of soil-grown plants members of the *Enterobacteriaceae* family were identified. Endophytes of the above-ground tissues especially that of the soil-grown plants were mainly Gram-positive bacteria.

Results of the seed germination test revealed that certain strains belonging to the genera of *Enterobacter, Brevibacillus, Delftia* and *Leclercia* had strong inhibitory effect on seed germination, while some species belonging to the genera of *Pseudomonas* and *Paenibacillus* had outstanding stimulatory effect.

4. CONCLUSIONS, SUGGESTIONS

More than 150 potential endophytic bacteria were isolated from two cultivars of *Capsicum annuum* var. *grossum* grown under soil and hydro-culture conditions. Comparison of the phenotypic and genotypic characteristics did not result unequivocal correlations, because in some cases isolates with identical molecular fingerprints showed high similarities on the phenograms, while in other cases both fingerprints of the same isolate were very different. Based on sequencing the 16S rRNA and *rpoB* genes some isolates could be identified at species or at least at genus level, but in other cases identification failed. In the latter case there is a chance of finding novel species, but exact identification requires further research work. Results of the germination tests showed occasionally that the potential endophytic nature of isolates belonging to the same species differed at strain level as different isolates of certain *Pseudomonas* species showed either inhibitory or stimulation effects.

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DEVELOPMENT AND ANALYTICAL DESCRIPTION OF A HUMAN *IN VITRO* DIGESTION MODEL

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SUMMARY

In vitro digestion models are widely used to study the structural changes, digestibility and release of food components under simulated gastrointestinal conditions. However, the results of in vitro digestion models are often different because of the difficulties in simulating the highly complex human digestion system. The in vitro models typically use the same digestion temperature, but the other parameters (applied enzymes, pH, digestion times and volumes) vary considerably and they are usually analytically not characterized. Therefore, our aim was to create a complex digestion model, which is modelled as accurately as possible the steps of human digestion and appropriate analytical techniques provide exactly characterized, reproducible results.

1. INTRODUCTION

In recent years there has been an increasing interest in development of *in vitro* digestion models to simulate gastrointestinal conditions and monitoring the structural changes in digestibility, bioavailability of bioactive components of foods, which are believed to benefit human health. Methods that simulate under laboratory conditions the gastrointestinal digestion process are used as a suitable alternative to *in vivo* assays; however the results of *in vitro* models are often different because of the difficulties in accurately simulating the highly complex gastrointestinal conditions. The *in vitro* models try to reproduce the physiological conditions in the mouth, stomach, and small intestine during mastication, digestion, and absorption. The physiological parameters of the gastrointestinal system and the composition of the digestive juices vary continuously due to the different environmental conditions and it is difficult to create a model which generally characterizes the human digestion. Most laboratories use a different method for modelling human digestion and the results of *in vitro* digestion models are often different (Hur, 2011; Oomen, 2002) and the efficiency of digestion is not characterized by appropriate means. Our aim was to detect the changes of different food matrices during the digestion process therefore we created a complex *in vitro* digestion model, which is modelled, as accurately as possible the steps of human digestion and appropriate analytical techniques provide exactly characterized, reproducible result. In our model the digestion is simulated in a simplified manner by applying physiologically based conditions, i.e. chemical composition of digestive fluids, pH and residence time periods typical for each compartment. The model describes a three step procedure simulating digestive processes in mouth, stomach and small intestine. The principle of this *in vitro* digestion model simulated the digestive processes under fasted conditions (Oomen, 2003). To mimic fed conditions some changes have been applied and have been described in detail (Versantvoort, 2004 and 2005). These models have been developed for testing the bioavailability of food contaminants and it was necessary to modify to be able to detect the changes of functional foods during the digestion process. In this study we describe the development of the first stage of digestion model, the selection of digestive enzymes.

2. MATERIALS AND METHODS

2.1. Enzymes, substrates and other reagents

During the development of the *in vitro* digestion model several commercial enzymes were used, the optimized enzymes were as follows: pancreatin from porcine pancreas (Sigma, P7545, 8xUSP specifications), pepsin from porcine gastric mucosa (Sigma, P7000, activity 666 U/mg), α-amylase from porcine pancreas (Fluca, 10080, activity 43.6 U/mg) and lipase from porcine pancreas (Sigma, L3126, activity 242 U/mg). The substrates used were water-soluble starch (Reanal), sunflower oil (Sigma) and sodium-caseinate (Sigma). All other chemicals for the digestive fluids were obtained from Merck, except for glucuronic acid (Sigma), bile (Sigma), mucine (Sigma) and glucosamine hydrochloride (Sigma).

2.2. *In vitro* digestion procedure

Digestive juices were prepared artificially and the composition of the various synthetic juices has been described previously in detail (Versantvoort, 2005), however several modifications were applied. The changes were as follows: glucose content removed from the gastric juice and the concentrations of several enzymes were halved in the gastric and duodenal juice (pepsin 1.25 g/l; pancreatin 4.5 g/l; lipase 0.75 g/l). To create a standard condition 4.5 g of the artificial food matrix (contained 1.5-1.5 g water-soluble starch, sunflower oil and sodium-caseinate in 1:1:1 ratio) per digestion was used. All digestive juices were heated to 37±2 ºC. The digestion started by adding 6 ml saliva to the standard food and was incubated for 5 min. The pH was set to 6.8. Then 12 ml of gastric juice was added, and the mixture was rotated for 2 hours. The gastric pH was between pH 2 and 3. Finally, 12 ml of duodenal juice, 6 ml bile, and 2 ml 1 M sodium-bicarbonate solution were added simultaneously, and the mixture was rotated for another 2 hours. The pH of the mixture was set to 6.5–7. All rotations were performed with a heated magnetic stirrer (200 rpm at 37±2 ºC).

2.2. Analytical methods

Several rapid analytical techniques were used to monitoring the starch, protein and lipid degradation. For analysis of proteins the digestion were stopped by changing pH significantly to inactivate the proteases (raising the pH to 10.0 in the case of pepsin and reducing the pH to 2.0 in the case of proteases in duodenal juice). SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) was carried out to evaluate the protein profile. The assay was performed according to standard protocols (Laemmli, 1970), using 17 % polyacrylamide gels and 10x10 cm glass plates. Samples (8 µl) were added to 2 µl sample buffer and 0.5 µl reducing agent (protein loading buffer pack, Fermentas) and incubated at 95 ºC for 5 min. Samples (10 µl/lane) were loaded on gels and then were run for 3 hours at 25 mA. Gels were stained ProSieveBlue Protein Staining Solution according to the manufacturer's protocol (Lonza). ProSieve QuadColor Protein Marker 4.6-300 kDa (Lonza) and ProSieve Unstained Protein Marker II 10-200 kDa (Lonza) were used as a wide range protein molecular weight marker.

Lipases hydrolyses the ester bonds in *triglycerides*, to form fatty acids and glycerol and monitoring the release of fatty acid is the most direct approach to determine lipase activity. The titration assay was used to determine the released free fatty acids using sodium hydroxide solution. The assay is based on the titration of pH drop associated with fatty acid formation. 5 ml samples originated from the *in vitro* digestion models were added to 5 ml acetone to stop

the digestion. Thereafter 2 drops of 1 % phenolphthalein were added to the samples and liberated fatty acids were titrated with 0.1 M sodium hydroxide.

To determine the degradation of the starch we used two different methods. The exact carbohydrate concentration values have been monitored by high performance liquid chromatography system (Shimadzu LCMS2010EV) using a Prevail carbohydrate column (250×4.6 mm, 5 µm; functionalized with amino groups) equipped with a guard column. A binary eluent system including water (A) and acetonitrile (B) was used in a gradient setup: t[minutes](B%):0(70)-20(60)-30(58)-32(70)-36(70)]. The injected volume of the sample solutions was 10 µl and the eluent flow was set to 0.8 ml/min. PL-ELS-2100 evaporative light scattering detector (ELSD - Polymer Laboratories) was applied to detect carbohydrate signals. For rapid testing of the samples reducing sugar content was determined by standard Luff-Schoorl method.

3. RESULTS

The efficiency of the most frequently used digestive enzymes purchased from different manufacturers was compared and the number of tested enzymes was the follow: pancreatin (3), pepsin (3), trypsin (2), chymotrypsin (1), α-amylase (4) and lipase (4). All enzymes originated from porcine pancreas or gastric mucosa (pepsins) with the exception of chymotrypsin (bovine pancreas), two α-amylases (*Bacillus subtilis* and *Aspergillus oryzae*) and one lipase (*Rhizopus oryzae*). The microbial enzymes were less effective than the porcine enzymes and it should be noted that most enzymes used for *in vitro* digestion studies are collected from omnivorous animals (usually porcine origin). Figure 1 A-C shows the digestion profiles of sodium-caseinate generated by several different enzymes and the main degradation products of starch samples (D).

The digestion efficiency of enzymes is only one factor what we need to study during the selection of the enzymes. Although well known that the manufacturers of enzymes use different sugars (mainly lactose) as additive it was surprising that the sugar content of certain enzymes were 80-90 % (w/w). The sugar content of the selected enzymes determined by HPLC was as follows: pancreatin (33.33 % lactose), α-amylase (23.29 % lactose), lipase (31.33 % lactose) and pepsin (74.38 % glucose). The digestive juices applied in the *in vitro* models contains usually a large amount of rough enzyme extracts (with several contaminating agents) because the purified and therefore more active and less contaminated enzymes are more expensive and the most purified enzymes are less realistic model of the gastrointestinal system. In the case of low activity enzyme products large quantities need to be used to achieve proper digestion efficiency, but this will increase the level of contaminants as well which may cause several problems. First, the SDS-PAGE analysis of the digestions enzymes resulted in the same electrophoretic patterns in the case of pancreatin, lipase and α-amylase (all originated from porcine pancreas) and we determined the protease activity of lipase (Sigma, L3126) and pancreatin (Sigma, P7545) are close to the same (Figure 1. E, F). The similar electrophoretic profiles of pancreatin, lipase and α-amylase suggest, that each enzyme have the same activity, but all possible cross-activities were not determined. Second, the high sugar content of enzymes greatly increase the sugar content of digestive juices (0.3 % or more) and this may cause problems during further applications especially in testing prebiotic substances. In order to reduce the sugar content of digestive juices we applied more concentrated enzymes than usually used in digestion models thus allow us to use smaller amounts with the same of better digestive efficiency. The sugar content of the optimized digestive juices was less than 0.08 %. Using the optimized enzymes not only reduced the amount of contaminants, but also increased the efficiency of digestion.

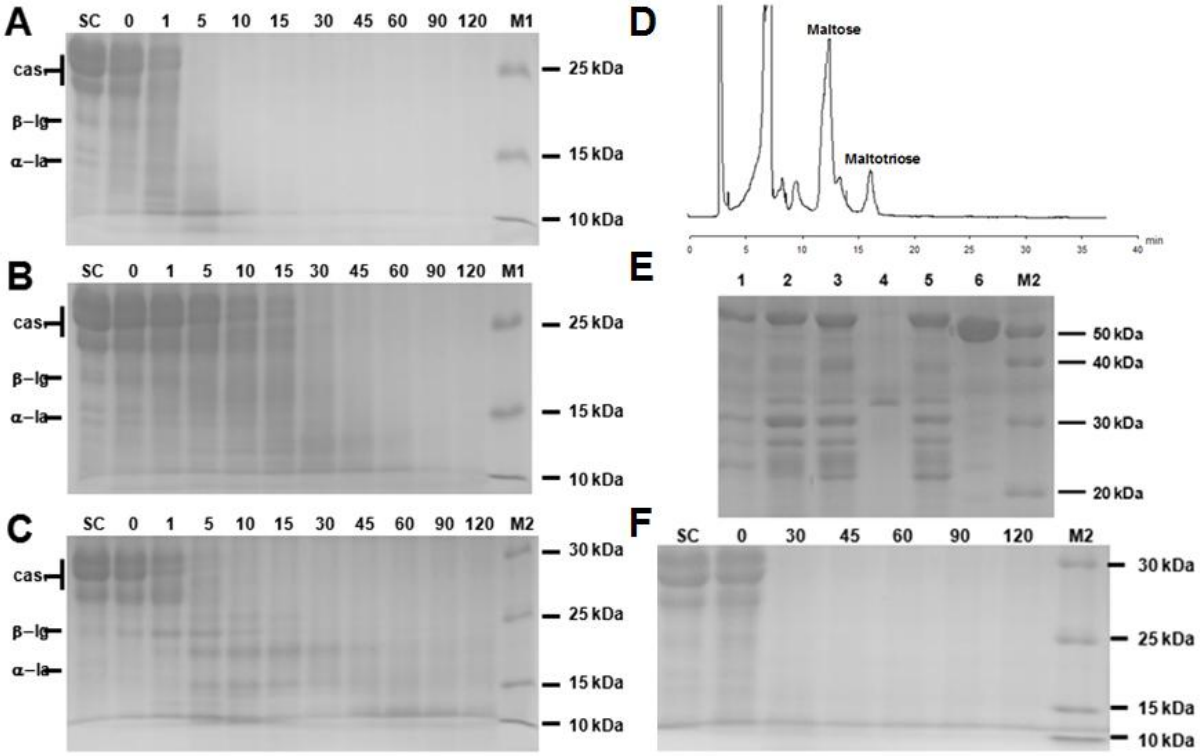

Figure 1. SDS-PAGE analysis of several digestions. Time based degradation of sodium-caseinate with 8xUSP pancreatin (A), 1xUSP pancreatin (B) and pepsin (C). SC: undigested sodium-caseinate, cas.: different casein components, β-lg: β-lactoglobuline, α-la: α-lactalbumine, 0-120: time of digestion (min), M1 and M2: protein molecular weight markers. D: The main degradation products of starch digestion determined by HPLC. E: Electrophoretic profile of several digestion enzymes (10 mg/ml). Lane 1: 1xUSP pancreatin, lane 2: 8xUSP pancreatin, lane 3: lipase, lane 4: microbial lipase, lane 5: α-amylase, lane 6: microbial amylase. F: Time based degradation of sodium-caseinate with lipase.

4. DISCUSSION

The main advantage of this *in vitro* digestion model is that it is easy to apply and several dozens of samples can be handled within a limited time. Analytical characterization of the digestion model allows continuous monitoring of digestion and the appropriate analytical techniques provide reproducible results.

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ANALYSIS OF FOOD SAFETY MANAGEMENT SYSTEMS IN SERBIAN FOOD PROCESSING INDUSTRY

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SUMMARY

Application of HACCP system in Serbia is compulsory since 2010 for all registered food processors. The multinational trading companies are not satisfied with the application of this system, they are also require other food safety management systems from their suppliers- small and medium sized companies. In our work we have analyzed which food safety management systems are required and ability of the food-processing companies to give answer for this challenge. We are also briefly introduced applied food safety systems.

1. INTRODUCTION

Nowadays the application of the food safety standards is like an entry ticket into the global food trade. (Mensah-Julien, 2010) Therefore the most of the food processing industry companies possess properly determined and formed quality management system.(Muñoz, 2002).

Businesses use private standards (beside their obligatory HACCP food safety system) today strategically, whether it is to gain access to new markets, to coordinate their operations, to provide quality and safety assurance to their consumers, to complement their brands, or to define niche products and markets (Hatanaka et al., 2005; Smith, 2009).

2. RESEARCH METODOLOGY

As primary research method, we analyzed used food safety systems in Serbia.

As secondary research methodology we have used questionnaire (in Serbian language). It contains open and closed clear questions, where respondents might be given answers according to the implementation and applying of food safety management systems. The survey has covered a total of124 small and medium sized companies, and has been conducting during 2012-2013.

Questionnaire contains questions for the representatives of small and medium sized companies according status of implementation food safety systems and their supplier status at multinational trading companies, as we show below:

Table 1: Information according to the food safety system and standards-questionnaire

Please tick the right answer below					
		We do not implemented	Implementation is in progress	Implemented	Certified
1.	HACCP system				
2.	EN ISO 22 000:2005				
3.	FSSC 22 000				
4.	IFS Food v.6				
5.	BRC				
6.	Are we supplier at any multinational trading company?				
	Yes	□	No	□	

Source: Own design, based on applied questionnaire

3. RESULTS AND DISCUSSION

3.1. The food safety system and standards

The following food safety system and standards are implemented in Serbian food processing industry:

- ***HACCP system,***

When we speak about the HACCP system, basically we thinking on the method of the FAO / WHO Codex AlimentariusCommission published the first version, which contains the HACCP (Hazard Analysis, Critical Control Point) system application's principles and practical rules. This procedure is basically theGeneral Principles Of Food Hygiene, CAC/RCP 1-1969", which was modified first in 1999, then in 1997 and finally in 2003.
The Hazard Analysis and Critical Control Point (HACCP) is a Food Safety Management System that is recognized in the international food safety community as a world wide guideline for controlling food borne safety hazards. (Dimitrios et all., 2013)

- EN ISO 22 000:2005,

The ISO 22 000:2005 standard was elaborated for the purpose of certification of HACCP. The standard is applicable for all the processes in food chain. This standard was elaborated by the ISO - International Organization for Standardization. The EN ISO 22 000:2005 international standard determines such a food safety management system requirements, which combines the following generally accepted, key elements what ensures food safety, along to the food chain, to the final consumer: mutual communication, system management, prerequisite programs, HACCP principles. Following the successful certifying the certification would be issued by the certification body. The certificate is valid for three years, along with successful annual supervision. (Kovács Sárkány- Kovács, 2013)

- FSSC 22 000,

The FSSC 22000 (Food Safety System Certification) is the newest, comprehensive food safety system created for the food manufacturer companies, which has been created in 2004 and the „Foundation for Food safety Certification" has been elaborated. Based on the EN ISO 22 000 food safety system, in the case of food manufacturers it is complemented by the BSI-PAS 220/ISO TS 22 002-1:2009 regulation. The BS-PAS 220:2008 such a prerequisite programs, which contains safety regulations exclusively for food manufacturers, since the BSI-PAS 223:2011 regulate the pre-condition programs related to the food packaging. Essentially this extension contribute to the better application of the ISO 22 000 standard. Those companies which have been already applied the ISO 22000 standard, need only one short inspection in order to prove that they perform the PAS 220, or the PAS 223 conditions (concerning to the hygiene etc.). The standard accepted by the Global Food Safety Initiative. The significance is that there are numerous customers who are accepted this certification from their suppliers, since the "simple" ISO 22 000:2005 certification is not enough.

-IFS Food.

The members of the German Retail Federation (HDE) and the French co-organization (FCD) have drawn up under the name of International Food Standard (IFS) the quality management and food safety standard concerning to the retailers own brand name of traded products.

Involve the HACCP food safety system elements, beside other standard requirements. The aim of this standard is to evaluate the suppliers' food safety and quality management systems by a uniform criteria system. (Kovács Sárkány- Kovács, 2013) The certificate is valid for one year.

3.2. Results of the research

Despite the fact that the HACCP system is mandatory for the whole food processing industry in Serbia (except for primary production), one part of the responding companies do not operate it (6 companies). These companies are not supply for the multinational food trading companies, neither exports their products. Distributions included in 1.figure.

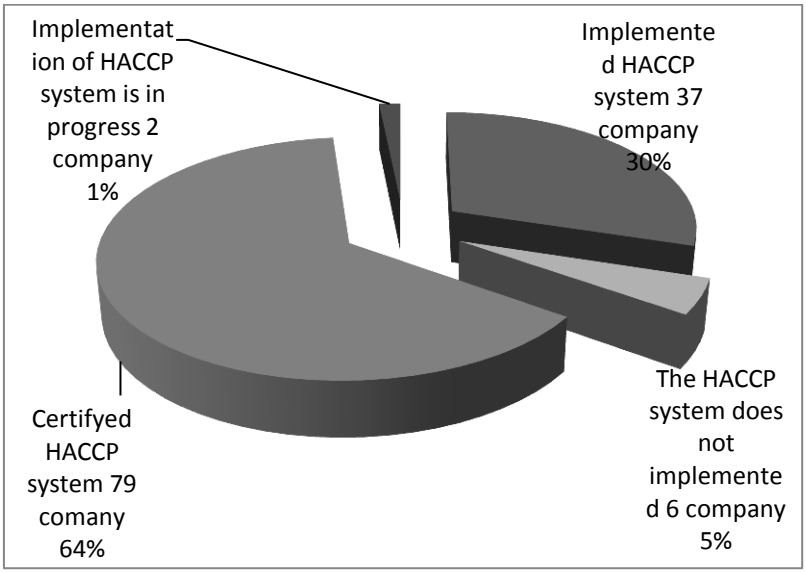

Figure 1: The application distribution of the HACCP food safety system at the analyzed companies

The whole of the companies does not implement any food safety standard (72%), as we showed in figure 2.

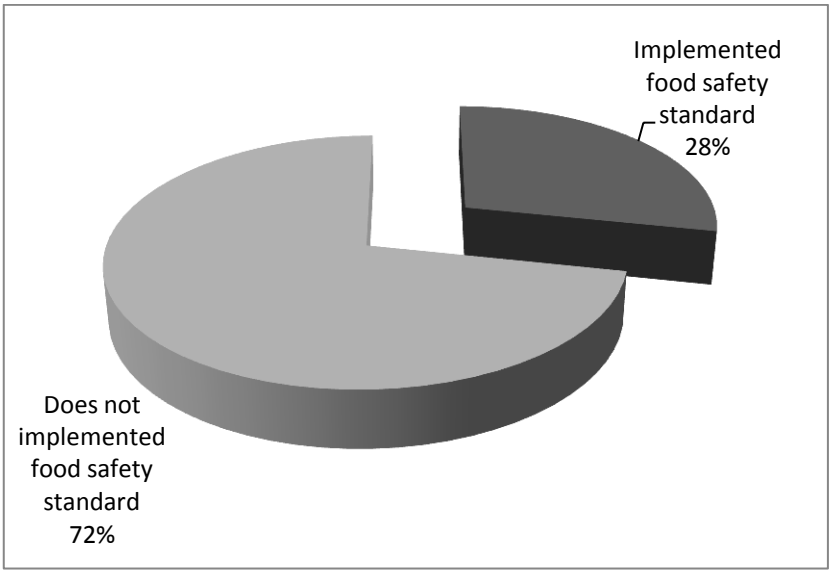

Figure 2: The application distribution of the whole food safety standards at the analyzed companies

The kind of implemented food safety standard and its distribution is showed below in figure 3.

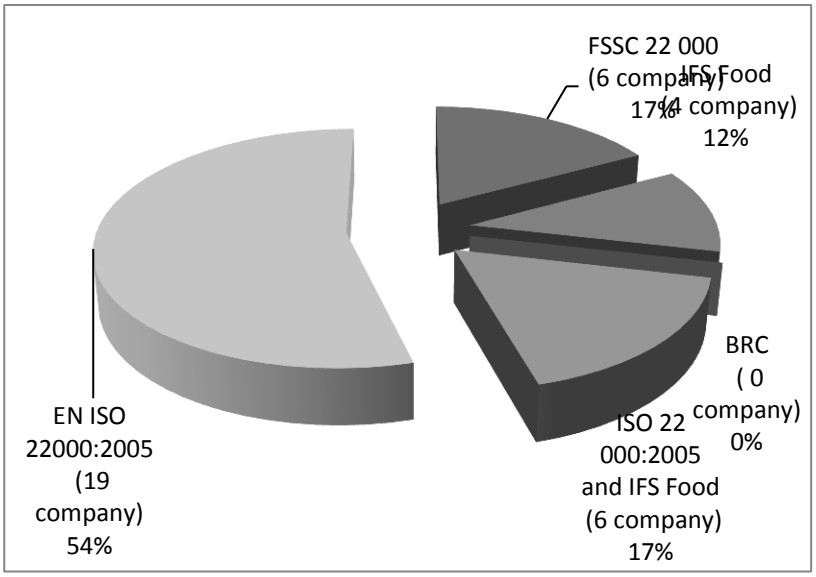

Figure 3: The application distribution of the food safety standards at the analyzed companies

Each company is a supplier of the multinational trade companies.

Same companies certifying their food safety system. In our sample 8 companies certified EN ISO 22 000:2005 standard, 4 companies certified FSSC 22 000 standard and 6 companies certified IFS Food standard.

4. CONCLUSION

Certain companies operate other food safety management systems, beside the HACCP food safety system, although the HACCP application is not yet complete.

Implementation of food safety standard is specifically for the suppliers of the multinational trade companies.

Clearly visible in this sense that, if the food-processing companies want to become suppliers for the multinational trading companies they are required to initiate food safety standards beside the mandatory applied HACCP system, and additionally on the customer's demand they have to certificate it too.

We think that this will be a big challenge for the examined small and medium food processing enterprises.

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HEALTH IMPACT OF INDUSTRIAL TRANS FATTY ACIDS (I-TFA)

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SUMMARY

I-TFA arising from the partially hydrogenated vegetable oils during the industrial process are very harmful to health by contributing to the development of systemic inflammation, vascular damages, insulin resistance, abdominal obesity, arrhythmias, diabetes and certain types of cancer as well. The recommendation of WHO maximizes the daily I-TFA consumption by 1 % of total energy intake which corresponds to maximum 2 g while. EFSA recommends as low as possible. Since 2009 NIFNS has been testing the I-TFA content in foods available on the domestic market. From the tested 729 fooditems, 159 samples (22 %) were found in which I-TFA exceeded 2 g / 100 g fat, and out of these samples there were 55 ones (35%) containing 5-10 g and 38 ones (24 %) over 10 g I-TFA / 100 g fat. Based on the results of the latest Hungarian Nutrition and Nutritional Status Survey the TFA intake could be 50,8 g at worst, increasing dramatically the cardiovascular risk. There is an urgent need for legislative measures.

COMPARATIVE INVESTIGATION OF PRE-AND PROBIOTIC PRODUCTS AVAILABLE ON THE HUNGARIAN MARKET, IN AN IN VITRO DIGESTION MODEL SYSTEM

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SUMMARY

*Our aim was to predict the potential physiological effects of some pre- and probiotic products, available on the Hungarian market. It was performed in an in vitro human gastrointestinal model system, which simulates the digestion process in mouth, stomach, small intestine and large intestine. The germ number of the probiotic products and the gastric and small intestinal transit tolerance of the individual bacterial species were determined. Prebiotic products were treated with enzimes, after that the digested samples were investigated in an intestinal model culture using two probiotic (*Bifidobacterium, Lactobacillus*) and two potentially pathogenic (*Clostridium, Bacteroides*) species. During the 48 hours of the incubation, the growth rates of the bacterial strains were determined regularly. In order to compare the prebiotic effect of the products the prebiotic index values were calculated.*

1. INTRODUCTION

The microbial community of the human colon has considerable effects on the well-being and health of the host and diet is an important factor in modulation of the gut microbiota's composition. In the normal gut microbiota, genera/species of microorganisms with potential health benefits are predominant in number over potentially harmful ones. Prebiotics are non-digestible, selectively fermented food ingredients, which beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health (Roberfroid, 2010) In the last few years, probiotic and prebiotic products are in a focus of an increased interest of the Hungarian consumers. These products are available as capsules, powders or beverages and offer several health promoting effects. Our goal was to assess the potential prebiotic effect of these widely used food supplements, on the human gut microbiome as well as the behavior of probiotic bacteria contents of these products in an *in vitro* human gastrointestinal model system. Two probiotic products and six prebiotic products were examined to predict their potential beneficial effects to the consumer.

2. MATERIALS AND METHODS

2.1. *In vitro* digestion procedure

The in vitro procedure simulates the human digestion in a simplified manner by applying physiological based conditions, i.e. chemical composition of digestive fluids, pH and residence time periods typical in the compartments of the gastrointestinal tract. The *in vitro* digestion comprises a three-step procedure simulating the digestion process in mouth, stomach and small intestine (Versantvoort, 2005; Hagens, 2008). Digestive juices are prepared artificially. Transit times, constituents and concentration of digestive juices and pH values chosen in different compartments are adopted from Oomen, 2003. This involves transit times 5 minutes and pH 6.8 in mouth, 2 hour and pH 2-3 in stomach, 2 hour and pH 6.5-7 in the small intestinal compartment, respectively. Experiments were performed at 37 °C according to the normal temperature of the human body. Constant mixing conditions are obtained by placing the vessels to a heating magnetic stirrer. Determination of the gastric and

small intestinal transit tolerance of the probiotic bacterial strains was performed according to Charteris, 1998 and Pan, 2009.

2.2. Tested products

The composition of the investigated probiotic and prebiotic products are shown in Table 1.

Table 1: Composition of the tested probiotic and prebiotic products

Product's name	Recommended daily intake	Inulin content	Other constituents
Probiotic yoghurt powder	15 g	11 %	probiotic *Lactobacillus acidophilus* culture
Probiotic capsule	2-3 capsule	30,5%	Probiotic *Bifidobacterium bifidum*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus* culture
Apple drops	6 tabletta / ~12 g	18 %	apple fibre 18 %, wheat fibre 26,6 %, fructose 8 %
Mineral drink	4 g	34,9 %	calcium, potassium, magnesium, zinc, iron, , manganese, copper, selenium
Tropic drink	30 g	18 %	wheat fibre 27 %, fructose 24,1 %, beta-carotene 0,3%
Peach drink	100 g	10 %	fructose 15 %, whey powder 64 %, whey proteins, beta-carotene
Apple drink	100 g	10 %	whey powder 78 %, whey proteins
Muesli drink	~ 15 g	-	cereal mixture 37,6 %, fructose 18 %, yoghurt powder 18%, milk minerals 6%, wheat germs 5%, wheat bran 5%

2.3. Prebiotic index

The prebiotic effect was quantified with the prebiotic index (PI) equation. (Palframan, 2003):

PI = (Bif/Total) – (Bac/Total) + (Lac/Total) – (Clos/Total)

where Bif is *bifidobacterial* numbers at sample time / numbers at inoculation, Bac is *bacteroides* numbers at sample time / numbers at inoculation, Lac is *lactobacilli* numbers at sample time / numbers at inoculation, Clos is *clostridia* numbers at sample time / numbers at inoculation and total is a total bacteria numbers at sample time / numbers at inoculation. The equation assumes that the increase in the population of *bifidobacteria* and / or *lactobacilli* is a positive effect while the increase in *bacteroides* and *clostridia* is negative. The bacterial counts were entered into the PI equation as log10 values.

2.4. Bacterial strains and growth conditions

Two probiotic strains were tested in mixed culture experiment *Lactobacillus casei* DSM 20011 and *Bifidobacterium longum* subsp. *infantis* DSM 20088 both supplied by the German Collection of Microorganisms and Cell Cultures. Whereas *Bacteroides fragilis* EKF 201 and *Clostridium perfringens* EKF 200 were used as representative pathogenic ones originated from Eszterházy Károly College, Egerfood Regional Knowledge Centre Collection of Microorganisms.

The bacterial strains were subcultured on blood agar plates for 48 hours. The bacterial strains were inoculated into the tubes containing the digested prebiotic products in an equal colony forming unit (CFU) as a mixed culture of probiotic and pathogenic bacteria. The tubes were incubated anaerobically at 37°C for 48 hours. For cell number counting samples were taken at 0, 24 and 48 hours from each tubes. The colony forming unit number was determined by plating dilutes on selective agars, Lactobacillus on MRS (de Man, Rogosa and Sharpe agar), Bifidobacterium on BSM (Bifidus Selective Medium Agar), Clostridium on TSC

(Tryptose Sulphite Cycloserine Agar), Bacteroides on BEA (Bile Esculin *Agar)*. Colonies were enumerated after 48 hours of incubation.

3. RESULTS

In our gastrointestinal model we investigated the gastric and small intestinal transit tolerance of some probiotic species derived by commercially available capsules and powders (Figure 1. and 2.).

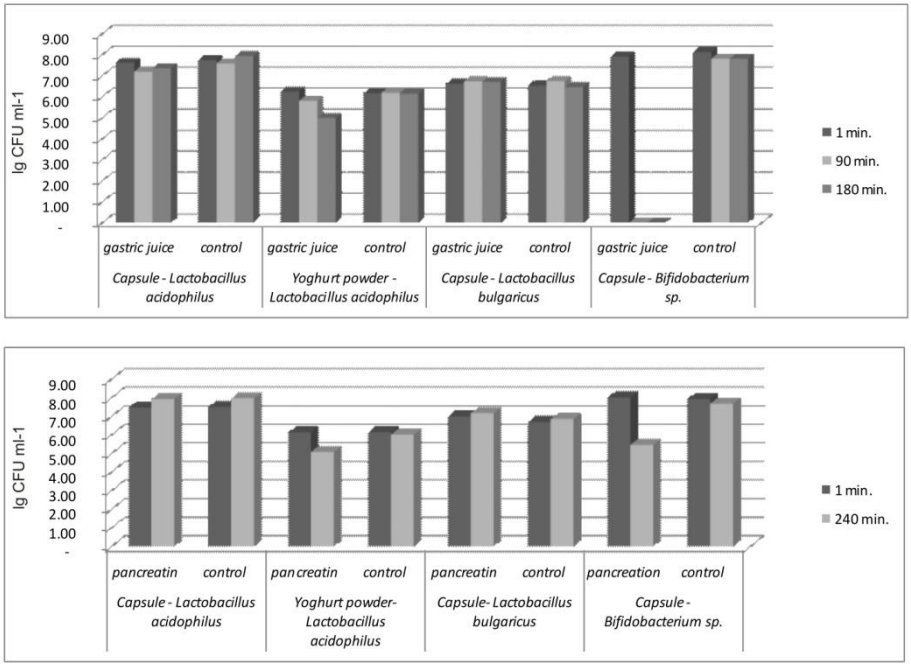

Figure 1: Gastric- and small intestinal transit tolerance of some probiotic species originated by probiotic capsule and yoghurt powder products

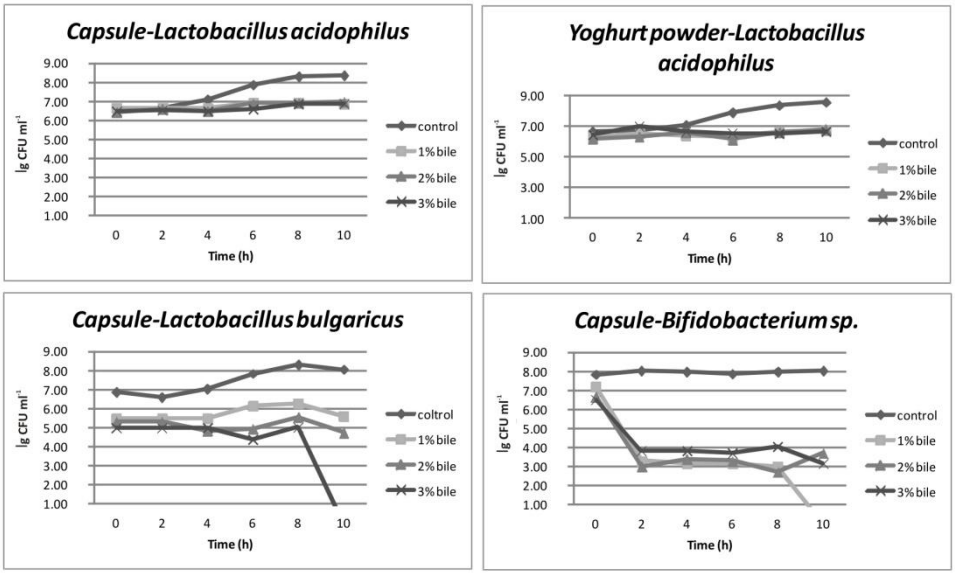

Figure 2: Bile resistance of probiotic species originated by probiotic capsule and yoghurt powder products

The three investigated *Lactobacillus* strain (Capsule: *L. acidophilus*, *L. bulgaricus*; Yoghurt powder: *L. acidophilus*) tolerated well the gastric juice (pepsin, HCl, pH = 2) and pancreatin (pancreatin, pH = 8), moreover they showed an appreciate level of survival in bile at 1, 2 and 3 % concentrations. The *Bifidobacterium* strain, from the capsule exhibited complete loss of viability during simulated gastric transit, and showed sensitivity against the bile salts, too. However, this strain was resistant against the pancreatic juice.

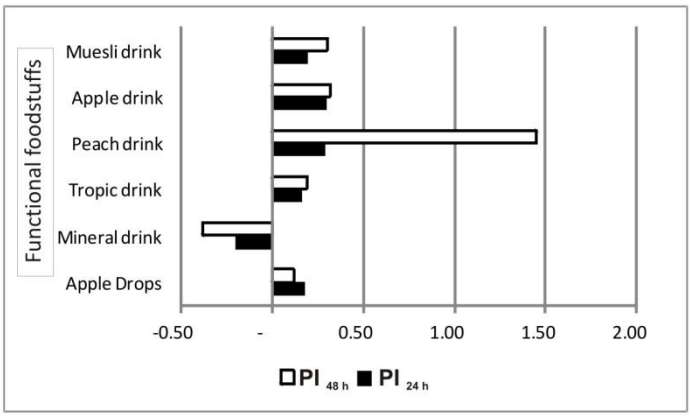

Figure 3: Prebiotic indexes of commercially available prebiotic products

Prebiotic index is a widely used quantitative tool to determine the prebiotic effect of different carbohydrates or foodstuffs. On Figure 3. are represented the Prebiotic indexes of the tested prebiotic products. The values were high in all tested samples, except the Mineral drink. Although this product also contains inulin, due to its buffer capacity, the probiotic *Bifidobacterium* and *Lactobacillus* species were not able to grow on it after longer exposure. Considering the calculated prebiotic index scores after 48 hours of incubation higher values were observed at the Peach drink following by Apple drink, Muesli drink, Tropic drink, Apple drops and Mineral drink.

4. DISCUSSION

The investigated probiotic products guarantee a required number of microorganisms, that may provide beneficial health effects to the hosts. However, in the absence of appropriate capsule material *Bifidobacterium bifidum* was not able to tolerate the gastrointestinal transit. Prebiotic food supplements stimulated selectively the probiotic model strains, and showed high Prebiotic index values.

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DEVELOPMENT OF AN ELECTRONIC TRACEABILITY SYSTEM SPECIALIZED FOR FUNCTIONAL FOODS

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SUMMARY

The market segment of functional foods is one of the few ones that has been growing continuously still in the last couple of years. Manufacturers use traceability systems the same way for functional foods as for regular foods. The primary aim in these cases is to ensure quick and efficient recall of batches having some food safety problems. At the same time, in the case of functional foods the consumers might require assurance that these products are not only safe but entirely preserve their health-promoting effects, as well. Nevertheless, data collection about these effects is not regulated by the law, so it is not possible to effectively recall products of bad quality or lost functionality. Our aim was to establish the informatics and technical basis of an electronic system that is able to follow unique characteristics of functional products.

In the first step the Failure Mode and Effect Analysis (FMEA) method was adapted and used for identification of such potential errors that might threaten unique features of functional food products. During evaluation, the risk factors were ranked and this ranking was used for identification of parameters to be recorded in the course of tracking.

Products with higher value are more exposed to falsification than regular products. In order to prevent falsification and decrease the resultant risks a neural network based identification protocol was developed, in which occurrence of representative components of the foods are compared to the previously recorded values, as a kind of fingerprint. For this purpose the Kohonen self-organizing map (SOM) algorithm is used.

High efficiency of electronic traceability systems can be used by peripheral devices that minimize errors of the input process. Therefore, elaboration of optimal combination of the active and passive forms of RFID technology is emphasized together with the recently introduced near-filed communication (NFC) technology; the latter technology might help to avoid recording of incorrect data and help to accomplish quick and efficient queries via modern mobile communication devices.

COMPREHENSIVE SCREENING OF POLYHPENOL CONTENT OF APRICOT FRUITS (*PRUNUS ARMENIACA*) CULTIVATED IN HUNGARY

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SUMMARY
Interest in polyphenol research is increasing, since a number of health-benefits have been associated these compounds. Consumption of edible plants provides polyphenols for human diet. In plants which these exist almost exclusively as various derivatives characterized by different bioavailability. Therefore, information on the polyphenol fingerprint of a plant food might be important when evaluating its nutritional value. In this study polyphenol fingerprinting of apricots cultivated in Hungary were carried out by HPLC-DAD-ESI-qTOFMS technique. Quantitation of major components was made by HPLC-DAD-ESI-TQMS method. In this work, the composition of polyphenols in apricot fruits, their qualitative and quantitative abundance were studied and the effects of harvest year were also considered.

6. INTRODUCTION

Nowadays, the scientific community increasingly recognizes that foods and beverages of plant origin including fruits and vegetables show some beneficial health-effects, *e.g.* inhibiting age-related diseases, especially cardiovascular diseases (CVDs) and certain types of cancer (Cook et al., 1996; Dauchet et al., 2006; Erlund, 2004). This effect has been associated with bioactive micronutrients such as polyphenols (e.g. flavonoids, phenolic acids and their derivatives (Holst et al., 2008; Scholz et al., 2007). Polyphenols are predominantly present in *planta* in the form of conjugates. Polyphenols are absorbed from the gastrointestinal tracts of humans and excreted partly either unchanged or most dominantly as metabolites in the urine and *faeces* (Cook and Samman, 1996; Crozier et al., 2010). It is shown in various reports that the different phenolic derivatives have different absorption properties as well as biological activity due to the type of their moiety (Clifford, 1999; Cook and Samman, 1996; Crozier et al., 2010; Dauchet et al., 2006; Erlund, 2004; Havsteen, 2002; Hollman et al., 1997, 1999; Morishita et al., 2001; Rice-Evans, 2004; Walle, 2004). Therefore the information on the polyphenol fingerprint of a plant food might be important when evaluating its nutritional value. Moreover, polyphenols are used by botanists for taxonomical classification (Havsteen, 2002).

Apricot is considered as a rich source of polyphenols, however; there are no comprehensive data available on the polyphenol fingerprint of apricots cultivated in Hungary. The Corvinus University of Budapest has an experimental orchard at Soroksár and an apricot breeding program conducted by the Department of Genetics and Plant Breeding, Corvinus University of Budapest. Thus a representative experiment could be carried out in order to study the composition of polyphenols in apricot fruit harvested in Hungary, their qualitative and quantitative abundance and also the effects of harvest year.

7. METHODS AND MATERIALS

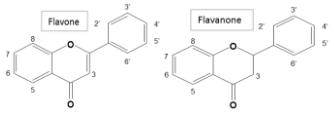
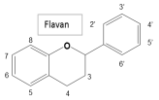
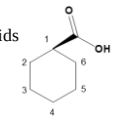
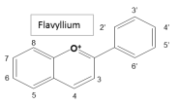
Polyphenol fingerprint of seven apricot (*Prunus armeniaca* L.) cultivars, including one traditional Hungarian cultivar (‘Gönci magyarkajszi’), three European cultivars (‘Ananasnyi ciurpinskii’, ‘Banaesa 4/11’ and ‘Goldrich’) and three hybrids (1/15, 7/1 and ‘Preventa’) were screened by high performance liquid chromatography coupled to a quadrupole time-of-flight mass spectrometer equipped with an electrospray ion source (HPLC-ESI-qTOFMS). Afterwards, a target compound method was utilized for using the multiple reaction monitoring (MRM) for quantification of the found major polyphenol compounds by a high performance liquid chromatography coupled to a triple quadrupole tandem mass spectrometer (HPLC-ESI-qTrapMS).

8. RESULTS

This study revealed that apricot, cultivated in Hungary is a rich source of several dietary polyphenols. With an in-house developed method approximately twenty different polyphenols were found in the samples, including different conjugates and isomers. The results of profiling are shown in Table 1. Eleven polyphenols were identified with reference materials, while only the type of conjugates could be defined for the remaining compounds. In most cases, differences were not detected among the cultivars regarding the quality and abundance of polyphenols. However, some special cases were also noted. For example, quercetin-hexoside-malonate – most probably the quercetin-3-*O*-glucoside-6”-malonate – was found only in hybrid 1/15. Cyanidin derivatives (kuromanin and keracyanin) - these compounds are responsible for the reddish colour of apricot fruit peels - where found in hybrids 1/15 and 7/1, ‘Gönci magyarkajszi’ and ‘Preventa’ cultivars. A naringenin-hexoside (most probably naringenin-7-*O*-glucoside since according to literature data this is the most abundant naringenin-hexoside in fruits) was found in almost all samples, but it was the most characteristic in ‘Gönci magyarkajszi’ fruits. Two different *p*-coumaroylquinic acids were found in the samples. These might be 3- and 5-*O*-caffeoylquinic acids (Phenol-Explorer). The 3-*O*-*p*-coumaroylquinic acid was found in all samples but 5-*O*-*p*-coumaroylquinic acid was characteristic only in ‘Banaesa 4/11’, ‘7/1’, ‘Gönci magyarkajszi’ and ‘Preventa’ cultivars. Two feruloylquinic acid isomers were identified, which might be 3-*O*-feruloylquinic acid and 5-*O*-feruloylquinic acid (Phenol-Explorer). The 5-*O*-feruloylquinic acid was characteristic only in ‘Gönci magyarkajszi’ and ‘Preventa’ fruits. Moreover, a dicaffeoylquinic acid was found in some of the studied apricot samples which have not been reported yet. It was only found in trace amounts in ‘1/15’, ‘Goldrich’, ‘Gönci magyarkajszi’ and ‘Preventa’. This compounds might be tentatively recognized as 3,5-*O*-dicaffeoylquinic acid because its retention time is the same as dicaffeoylquinic acid had in green coffee samples.

It can be concluded that there were no significance differences in the polyphenol composition of apricot fruit samples due to the harvest year effect.

Table 1. Polyphenol composition in fruits of apricot genotypes cultivated in Hungary

Polyphenols	Subclass	Name	Substitution	Elemental composition	Exact mass
Flavonoids	Flavonols	**Quercetin-3-*O*-glucoside**	**3, 5, 7, 3'-OH; 3-*O*-glucoside**	**$C_{21}H_{20}O_{12}$**	**464.0955**
		**Quercetin-3-*O*-glucoside-6"-acetate**	**5, 7, 3'-OH; 3-*O*-glucoside-acetate**	**$C_{23}H_{22}O_{13}$**	**506.1060**
		Quercetin-hexosyl-malonate	3, 5, 7, 3'-OH; *O*-hexosyl-malonate	$C_{23}H_{22}O_{15}$	550.0959
		Kaempferol- 3-*O*-rutinoside	**5, 7, 4′-OH; 3-*O*-rutinoside**	**$C_{27}H_{31}O_{15}$**	**594.1585**
		Quercetin-deoxyhexosyl-hexoside	5, 7, 3',4'-OH; *O*-deoxyhexosyl-hexoside	$C_{27}H_{30}O_{15}$	610.1533
		Rutin	**5, 7, 3',4'-OH; 3-*O*-rutinoside**	**$C_{27}H_{30}O_{16}$**	**610.1534**
	Flavanones	Naringenin-hexoside	5, 7, 4'–OH; *O*-hexoside	$C_{27}H_{32}O_{14}$	580.1792
	Flavanols	**(+)Catechin**	**3, 5, 7, 3', 4'-OH**	**$C_{15}H_{14}O_6$**	**290.0790**
		(-)Epicatechin	**3, 5, 7, 4', 5'-OH**	**$C_{15}H_{14}O_6$**	**290.0790**
	Anthocyanidins	**Kuromanin**	**5, 7, 4'-OH; 3, 5-OCH_3; 3-*O*-glucoside**	**$C_{21}H_{21}O_{11}$**	**449.1079**
		Keracyanin	**5, 7, 4'-OH; 3, 5-OCH_3; 3-*O*-rutinoside**	**$C_{27}H_{31}O_{15}$**	**595.1658**
Hydroxycinnamoylquinic acids		*p*-Coumaroylquinic acid	1, 3, 4, 5-OH; *O*-*p*-coumaroyl	$C_{16}H_{18}O_8$	338.1002
		**Neochlorogenic acid**	**1, 4, 5-OH; 3-*O*-caffeoyl**	**$C_{16}H_{18}O_9$**	**354.0951**
		Cryptochlorogenic acid	**1, 3, 5-OH; 4-*O*-caffeoyl**	**$C_{16}H_{18}O_9$**	**354.0951**
		Chlorogenic acid	**1, 3, 4-OH; 5-*O*-caffeoyl**	**$C_{16}H_{18}O_9$**	**354.0951**
		Feruloylquinic acid	1, 4, 5-OH; *O*-feruloyl	$C_{17}H_{20}O_9$	368.1107
		Dicaffeoylquinic acid	1, 3, 4, 5-OH; di-*O*-caffeoyl	$C_{25}H_{24}O_{13}$	516.1268

The major polyphenols included the following substances: neochlorogenic acid, catechin, chlorogenic acid, epicatechin, rutin - these were found in high concentration - nevertheless quercetin-3-*O*-glucoside, kaempferol-3-*O*-rutinoside and quercetin-3-*O*-glucoside-6”-acetate were determined at low concentration. These eight polyphenols were found both years in.

Table 2. The concentration of major polyphenols in the studied apricot genotypes

Apricot species	Neochlorogenic acid		Catechin		Chlorogenic acid		Epicatechin		Rutin		Quercetin-3-*O*-glucoside		Kaempferol-3-*O*-rutinoside		Quercetin-3-*O*-glucoside-6"-acetate	
	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011
	mg/100g dry weight		mg/100g dry weight		mg/100g dry weight		mg/100g dry weight		mg/100g dry weight		mg/100g dry weight		mg/100g dry weight		mg/100g dry weight	
1/15 hybrid	5,26	6,27	0,04	0,26	0,84	0,75	0,12	0,98	0,56	1,92	0,83	4,14	0,38	0,64	0,95	5,01
7/1 hybrid	8,26	10,46	1,04	1,33	7,23	5,15	4,79	10,07	1,58	3,94	<0,01	<0,01	0,95	2,03	0,90	3,24
Ananasnyi ciurpinskii	2,08	3,10	0,42	0,36	1,72	1,52	1,84	9,06	1,98	3,22	<0,01	<0,01	1,70	1,54	2,91	5,70
Banaesa 4/11	9,09	22,39	0,75	1,90	2,51	5,63	10,55	16,85	1,62	4,06	1,52	3,77	1,61	2,38	1,92	7,36
Goldrich	8,57	17,28	0,33	1,55	4,40	5,68	4,15	32,09	1,48	5,40	0,56	2,93	1,03	2,72	1,18	5,63
Gönci magyarkajszi	2,56	8,94	0,28	3,10	1,65	2,92	4,62	16,07	1,32	6,03	1,18	2,31	1,36	1,60	1,22	2,30
Preventa	62,50	64,50	5,49	22,27	12,62	12,72	4,32	5,61	3,24	1,78	1,27	1,34	3,64	1,42	0,41	0,39

The results of quantitative analysis of apricot fruit samples are presented in Table 2. Data show that concentrations of most of the eight polyphenols were similar in both years in ‘Preventa’ fruits. Interestingly, this cannot be said for other cultivars, in which 1.5 to 2 times differences in concentration were measured. These differences can be explained by the effect of harvest year. The climatic conditions of these two years were different. According to the results, harvest year had an effect on the quantity of polyphenols.

It can be seen that ‘Preventa’ is a special genotype. It was identified to accumulate total polyphenolics contents significantly higher than any other apricots (Hegedűs et al., 2010). Most components were found in this cultivar in the highest concentration. Fruit of this genotype was the richest source of polyphenols. The sample with the lowest polyphenol content in this study was hybrid 1/15, which contained polyphenol forms in quantities one order of magnitude lower than ‘Preventa’.

9. CONCLUSION

In this work, the composition of polyphenols of apricot fruits were investigated, their qualitative and quantitative characteristics and the effects of harvest year on polyphenol contents were studied. Twenty different polyphenols were found to be abundant in apricots. Eleven polyphenols were identified based on reference materials and the major eight polyphenols were also quantified. Genotype 'Preventa' turned out to be the richest source of polyphenols. Our results suggest that harvest year did not influence the qualitative composition of polyphenols; however, climatic conditions may cause 1.5-2-fold differences in polyphenol concentrations among specific harvest years.

ACKNOWLEDGMENT: *This work was supported by the OTKA-PD 100506 and OTKA K84290 projects and A. Hegedus is grateful for receiving a János Bolyai Scholarship, HAS.*

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EFFECT OF PRODUCTION TECHNOLOGY ON ANTINUTRITIVE COMPONENTS IN TUBERS OF POTATO VARIETIES

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SUMMARY
We have investigated the Total Glycoalcaloid (TGA), nitrite and nitrate content of some Hungarian and foreign potato varieties in relation to the effect of different combination of fertilisers and green manure, late blight management strategies (non, programmed or prediction based spraying) and irrigation regime for three years. The Hungarian varieties have exotic potato species like S. acaule, S. demissum, S. stoloniferum, S. vernei, S. tub. ssp. andigenum in their genetic background as source of resistance genes. Fertilisiers or irrigation were found not to have an effect on the level of glycoalkaloids and nitrate content; which were influenced mostly by the genotype and season. In conclusion the absolute amount and changes of different antinutritive components of potato tubers were influenced differentially by the technology, genotype and season in a complex manner.

1. INTRODUCTION

Potato is one of the most important staple foods and it plays a significant role in human diet worldwide (FAO, 2008). Its intensive cultivation assumes the operation of effective plant nutrition and plant protection systems. The use of varieties having a wide range of adaptability to diverse environmental factors (Hassanpanah, 2010), good nitrogen use efficiency (Gholipouri and Kandi, 2012), resistance against most important pathogens and pests (Forbs 1999) are prerequisites for successful potato production.

From the human nutrition point of view occurrence of several types of antinutritive components, such as steroidal glycoalkaloids, threaten consumer's health (see review by Nema et al. 2008, Friedman 2009). The glycoalkaloid content in tubers is affected by the genotype, climate, production technology, storage time, sprouting and exposure to light and heat.

Nitrogen fertilisation can cause significant increase in nitrate and nitrite content of tubers but the genotype and production technology may have effect on the concentration of these compounds (Augistin et al. 1977, Hamouz et al. 2005). Nitrogen is absorbed by plants in the form of either ammonium (NH_4^+) or nitrate (NO_3^-), depending on the species, cultivar, age and soil conditions (Rao, 2000; Greenwood, 1986). Nitrate accumulation in plants is a natural phenomenon resulting from uptake of the nitrate ion in excess of its reduction and subsequent assimilation.

The Expert Committee of FAO/WHO determined the Acceptable Daily Intake (ADI) value as 5 mg sodium-nitrate, and 0,2 mg sodium-nitrite/ body weight kg. Consumption of high levels of nitrate may cause health problems, for example methemoglobinemia in babies (Rao, 2000) and some cancers (Mensinga et al., 2003; Forman et al, 1985).

The objective of the present study was the comparison of some pathogen resistant Hungarian and in general pathogen sensitive foreign potato varieties regarding internal components of their tubers such as steroidal glycoalkaloids, nitrate and nitrite content grown under different farming practices. The investigation of seasonal effect (3 years) was also aimed

2. MATERIALS and METHODS

2.1. Plant materials and growth conditions

Potato varieties of University of Pannonia (Balatoni rózsa, Rioja, Vénusz, White Lady) showing complex resistance to potato viruses, fungi and bacteria and pathogen susceptible Dutch varieties (Laura, Red Scarlet, Desiree, Cherie, Franceline, Natasha, Saline) were used in the experiments. Varieties were cultivated at two locations (Komárom and Solt, Hungary) for three years (2010-2012) in four replications. Soil quality was determined by official soil sampling. The pH of soil varied between 6,8-7,2.

Methods:
Irrigation:
I. Non
II. + 90 mm irrigation water
Late blight control strategy:
I. Non
II. Programmed application
III. Prognostic application
Fertilization:
MT1. Fertliser 1 (N 50 + 200, P2O5 150, K2O 300 kg/ha)
MT1+Z. Fertiliser 1 + Green manure
MT2. Fertiliser 2 (N 100 + 200, P2O5 150, K2O 300 kg/ha)
MT2+Z. Fertiliser 2 + Green manure

2.2. METHODS

Potato processing: Potatoes were washed, peeled (2 mm thickness), crushed by chopper (Philips HR 1392). All samples were then freeze dried and subjected to further analysis.

Glycoalkaloids were cleaned on SPE and analysed by a RP-HPLC procedure using a GraceSmart RP C18 cloumn, isocratic elution and were detected at 194 nm using PDA. (Tömösközi-Farkas et al; 2006)

Determination of nitrate and nitrite content

Measurements were carried out with the standard method of AOAC Official Method 993.03 (2003) (Determination of nitrite and nitrate content; Molecular absorption spectrometric method).

Statistical analysis: for evaluating data paired t-probe was used (Microsoft Excel).

3. RESULTS

As the Figure 1. shows there were no significant differences in TGA content regardless of applied spraying strategies in the first two years. (Solt experiment in 2010 could not be evaluated due to flooding.) Inverse effect was observed between the fungicide use and TGA content in 2012. Higher number of fungicide application (programmed spraying) elevated the TGA content of tubers. The highest values were measured in tubers from non treated plots. This phenomenon could originate from the cumulated effect of biotic and abiotic stress caused by P. infestans infection and severe drought period of that year.

No correlation was observed between irrigation and TGA content of tubers (Figure 2). As the statistical analysis proved the differences were not significant and consequent. However the average TGA content in tubers of all varieties were lower in samples from Komárom compared to Solt.

Results regarding fertilization methods can be seen in the Figure 3. No significant difference was observed between treatments but higher dose of N (Fertilizer 2, MT2+Z) resulted a lower TGA content in Red Scarlet, Katica, Laura, Rioja and Balatoni Rózsa in 2012.

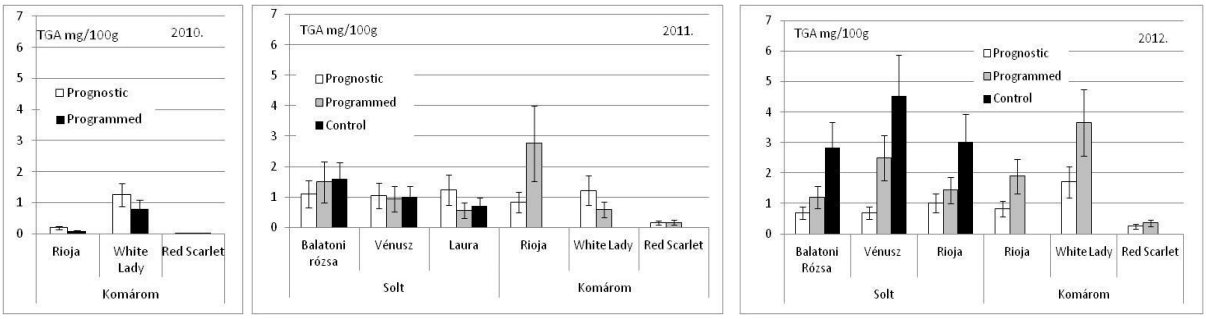

Figure 1: Effect of late blight control strategy on TGA content in potato Varieties

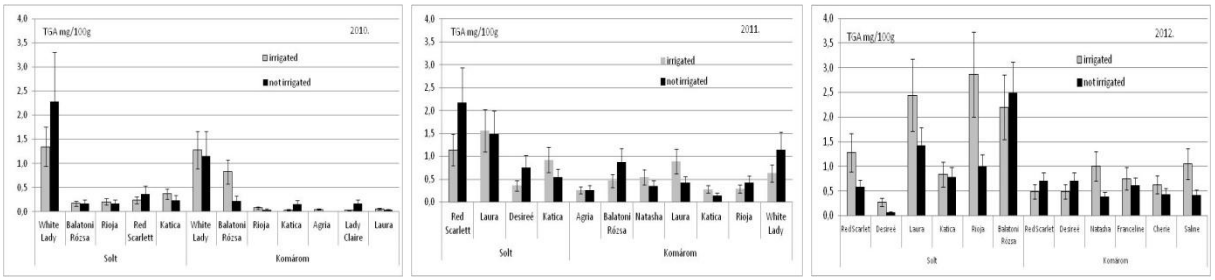

Figure 2: Effect of irrigation on TGA content in potato Varieties,

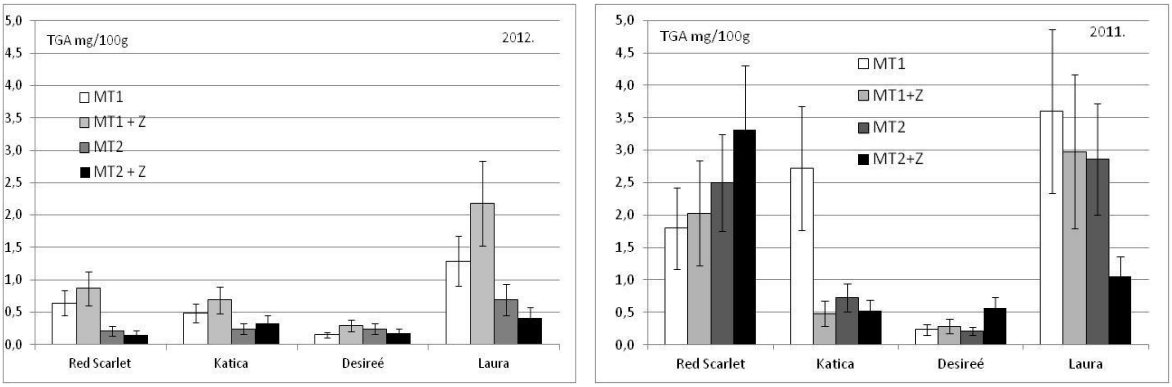

Figure 3: Effect of fertilization on TGA content in potato Varieties (MT1 – Fertilizer 1; MT1+Z – Fertilizer 1 + green manure; MT2 – Fertilizer „; MT2+Z – Fertilizer 2 + green manure)

The effect of irrigation and the fertilization were investigated on the concentration of nitrite and nitrate in tubers.

The nitrite content of tubers was lower than 0.1 mg/kg except in a few cases and there were no significant differences under the various circumstances (data not shown). As the Figure 4. shows there were no significant differences in nitrate content regardless of applied irrigation treatments in the first two years (experiments in 2010 could not be evaluated due to flooding at both locations.). Nitrate content was primarily influenced by the genotype.

Results regarding fertilization methods can be seen in the Figure 5. No significant difference was observed between treatments (Fertilizer 2) but the mean concentration of nitrate was two times higher in 2012 than 2011 in case of each varieties emphasising the stronger effect of the genotype and season.

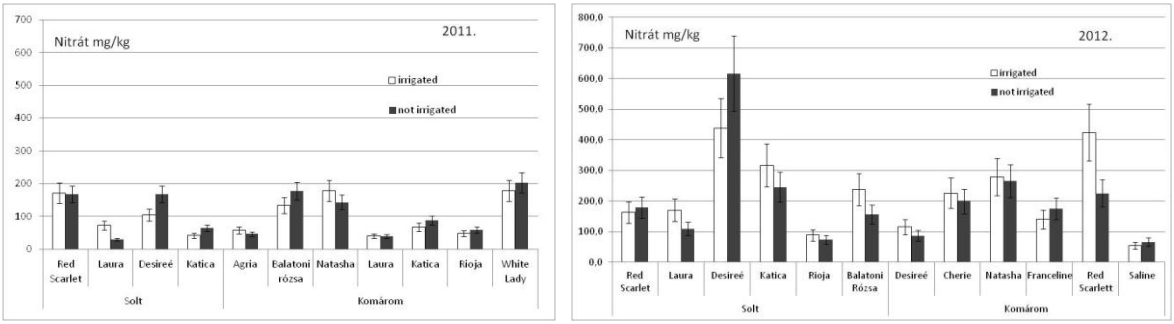

Figure 4: Effect of irrigation on nitrate content in potato Varieties,

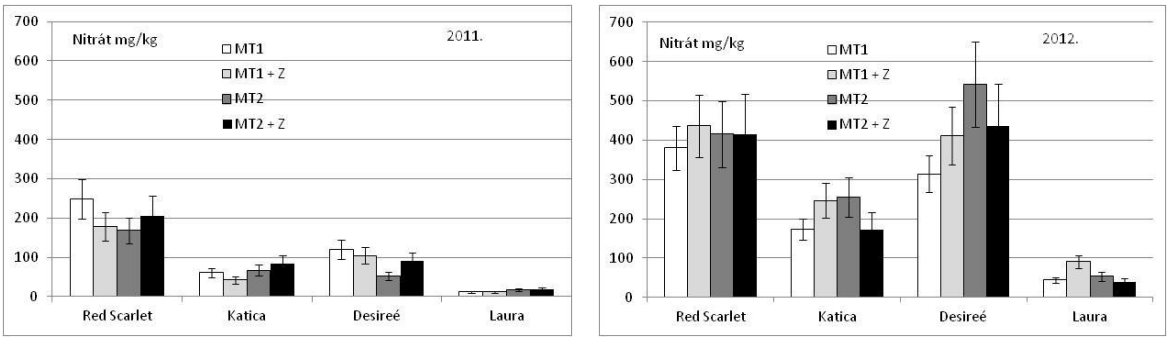

Figure 5: Effect of fertilization on nitrate content in potato Varieties (MT1 – Fertilizer 1; MT1+Z – Fertilizer 1 + green manure; MT2 – Fertilizer „; MT2+Z – Fertilizer 2 + green manure)

4. CONCLUSIONS

Based on our results we can state that under examined circumstances the genotype had the highest effect on tubers TGA and nitrate content that can be significantly modified by seasonal effects but not by applied agrotechnical methods like fertilisation, fungicide use (late blight control) or irrigation.

ACKNOWLEDGEMENT: *The financial support of the NFÜ (Burg009 project) is greatly appreciated.*

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NITRITE IN THE MEAT PRODUCTS

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SUMMARY

One of the most important additives of meat products in sodium-nitrite. It has bacteriostatic and sporastatic effect, inhibits outgrowth of microbes, first of all of Clostridium botulinum spores causing botulism, consequently it exhibits a significant affect in terms off food safety. In addition it forms the typical pink colour of meat products, has antioxidative effect and inhibits rancidity, too. It has favourable effect on sensory characteristics, chiefly on flavour and plays an important role in formation of typical cured flavour. Besides these important features concerning food safety and quality nitrite in higher concentration exhibits health risk. For this reason in the last decades the nitrite received a special attention in the media, food research and quality control, too.

1. INTRODUCTION

Sodium nitrite is probably the most important additive in meat industry having been used since more than 200 years. Exact history of its use is not known. Common salt used for preservation of meat was likely contaminated with salpetre (potassium nitrate). Meat prepared with this substance stayed nice pink in color and became characteristically flavored. This effect of salpetre was recognized in the 1800-s and they began to make use of it. Polenski in 1891 detected that nitrate can be transformed to nitrite as a result of bacterial metabolism, while Lehman in 1899 stated that characteristic pink color of cured meat is caused by nitrite not by nitrate. Haldene discovered in 1901 that this pink color is formed as a result of a reaction between nitrogen oxide and meat pigments (Cassens, 1990). These findings contributed to direct applications of nitrite making nitrate-nitrite transformation superfluous. Since nitrite *per se* is poisonous, it can be used only in form of curing salt, which is a 99,5% NaCl and 0,5% $NaNO_2$ blend. This way overdosage can be avoided (Incze, 1995).

Nitrite has several functions. It is bacteriostatic and sporostatic, inhibits growth of microbes, mainly that of *Clostridium botulinum* (causing botulism) by inhibiting outgrowth of its spores, exhibiting thus important food safety feature (Roberts and Smart, 1974). In addition it plays a decisive role in characteristic pink color formation of meat products (Sebranek and Bacus, 2007), has antioxidative effect, inhibits rancidity, prevents formation of WOF (Warmed Over Flavor) (Fox, 1987). It has a favorable effect on sensory value, first of all on flavor, in formation of characteristic cured meat flavor. In higher concentrations nevertheless it has a disadvantageous, health risk property: on the effect of inadequate technological and culinary practice nitrosamines can be formed from nitrite (Cassens and Hotchkiss, 1988). For this reason its use is strictly regulated.

Since scientific and practical interest for nitrite has been ever since vivid, and since information misleading the consumers can be heard and read rather often, I considered very important to launch and evaluate a series of experiments, that investigate the effect of different nitrite concentrations on chemical, microbiological and sensory characteristics with heat treated and non heat treated meat products equally.

2. MATERIAL AND METHOD

The aim of my experiments heat treated models (Bologna sausage) were prepared with different nitrite concentrations (0, 50, 100, 150 mg/kg) following the domestic technology. Bologna sausage was stored for 90 days at 4 °C. Following parameters were measured during storage:

- Chemical: pH, moisture, nitrite and nitrate content, total pigment content, nitroso pigment content, reddening %, rancidity
- Microbiological: total aerobic count
- Color: lightness, red color intensity, hue and stability of color during light exposure
- Texture: hardness, chewing energy need and elasticity
- Sensory: color, odor (aroma), flavor, texture and overall acceptance

3. RESULTS

3.1. Nitrite and nitrate content of ingredients (meat, seasonings, vegetables)

These ingredients also contain nitrate. Source of meats' nitrate content is animal feed and water. Nitrate content of beef is higher than that of pork or poultry. Also seasonings have nitrate content, the highest being in paprika (red pepper), as a consequence nitrate content of meat product can be elevated by 15 mg/kg. High nitrate content is typical for flavor enhancers, too. Product development in meat industry involves application of vegetables in an ever increasing manner aiming at healthier nutrition thanks to their high fibre content. At the same time they increase nitrite content of the product (e.g. addition of 15% chive increases nitrite content of meat product by 150 mg/kg).

3.2. Effect of amount of added nitrite on characteristics of Bologna sausage

Concentration of sodium nitrite in meat batter decreased significantly starting immediately after addition and is further reduced during heat treatment. The higher the initial concentration the higher its residual value, evidently. This concentration decreases until 50th day of storage when reaching a steady state. This fact is important to know in case of comparative tests, since values will differ depending on timing of measurement (being in unstable range results are not comparable).

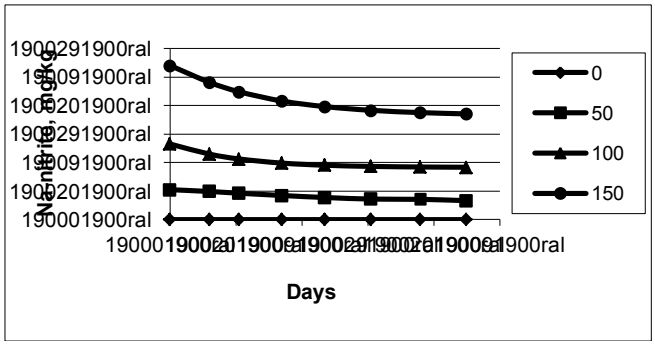

Figure 1: Sodium-nitrite content during storage

During manufacturing part of nitrite is transformed to nitrate, no such transformation takes place in final product. The higher the initial nitrite content the higher the nitrate formed. Nitrate content in final product decreases during storage until day 50, and similarly to nitrite the system becomes stable with a limit.

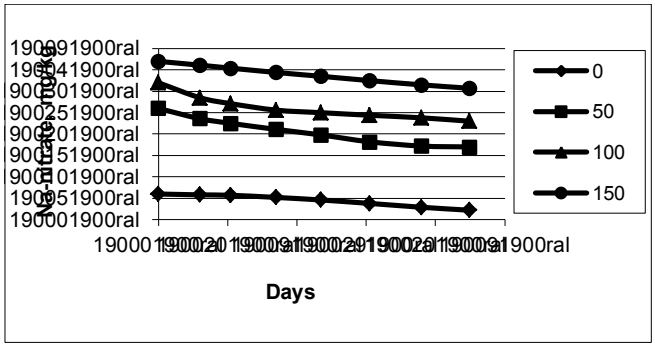

Figure 2: Sodium-nitrate content during storage

As experiments showed amount of nitrite above a certain level does not influence the amount of total pigment: same amount is formed affected by 50 or 150 mg/kg sodium nitrite. The same is true for the content of nitrosopigment. Both pigment contents are reduced somewhat until the 34th day of storage reaching then a stable value, reddening % is this way constant during storage. Bologna sausage without added nitrite also contains nitrosopigment explained by the fact that nitrite content of meat itself makes a slight nitrosopigment formation possible.

When analyzing rancidity, evidently no nitrite effect was detected in Bologna sausage stuffed in oxygen-proof casing.

In case of suitable pasteurizing heat treatment added nitrite amount did not influence microbiological status of meat product.

Analyzing the results of color measurements it was stated, that Bologna sausage without added nitrite was the most pale, the least red in terms of red color intensity and color. When increasing nitrite concentration color of the product turns red and deeper. No differences were found between Bologna sausage samples with 100 and 150 mg/kg added nitrite. Color characteristics hardly change during storage. As it was shown 50 kg/kg added nitrite is sufficient for proper color formation.

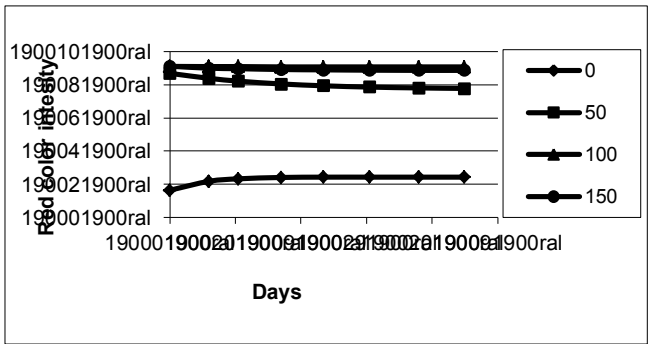

Figure 3: Red color intensity during storage

During measurement of texture it was stated that samples with nitrite were harder and more elastic. Nitrite content and time did not influence texture.

Bologna sausage samples without added nitrite proved sensoric objectionable mainly because of their greyish color, but other characteristics (aroma, flavor, texture, overall acceptance) turned out of lower grade. Increasing of amount of added nitrite on the other hand did not cause significant improvement above a certain level in these characteristics. Accordingly 100 mg/kg of added nitrite is fully effective for ensuring proper sensory characteristics of Bologna sausage. Values of sensory characteristics gradually decreased during storage resulting in loosing of mainly flavor characteristics, the chief sensory property. It was stated that flavor deteriorations takes place also without lipid oxidation, caused by different (mainly protein) degradation. This phenomenon can be detected already at day 30 during 90 days storage, sensory value is halved by then.

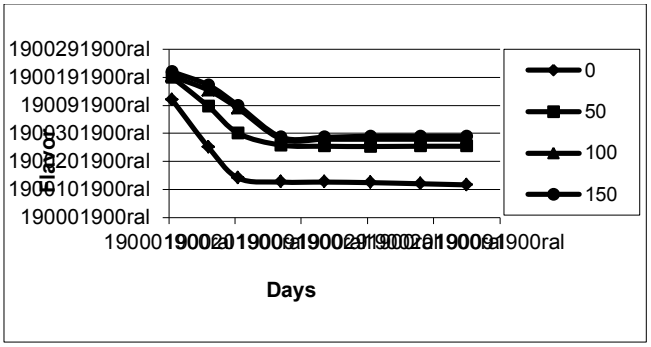

Figure 4: Flavor intensity during storage

This should be taken into consideration by the food chains that require production of items with a shelf-life of longer-than-ever.

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CLASSIFICATION OF FUSARIUM INFECTED WHEAT SAMPLES WITH NIR

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SUMMARY

Under certain conditions the fungus Fusarium produces a toxin, which can cause serious public and animal health problems. Samples (2010 rainy vintages experimental varieties) were provided by the Martonvásár Crop Research Institute. The samples were classified in four groups according to their infection status.
The research was conducted with METRINIR 10-17 ST-type device in transmission mode (wavelength 700-1700 nm, 2 nm gate distance). In each group 40 wheat kernels were tested, then the grain samples were grinded to grit to achieve better homogeneity. The grits were then measured with 15 repetitions per group. Our goal was to clearly separate the groups of the infected wheat samples, to find correlation between infection level and nutritional content. Our finding was, that the protein content and starch content of the fungus Fusarium infected wheat decreased significantly, according to the infection level.

1. INTRODUCTION

The infection of wheat with *Fusarium* is a well-known risk. It means a loss in quality and quantity of wheat, and the bakery value of the flour made of *Fusarium* infected wheat is also lower. Under given circumstances the fungus can produce a toxin, which represents potential risk for the public health.

The aim of this research was to prove, whether or not the near infrared spectroscopy (NIR) is suitable to give information about the *Fusarium* infections of different wheat samples.

Previously other researchers showed, that the NIR is suitable to follow the changes in the protein content of different food stuffs (Lin et al., 2004; Ellis et al., 2002; Kaffka and Martin 1985; Dalmadi et al., 2007; Wehling et al. , 2006).

2. MATERIALS AND METHODS

2.1. Wheat and flour samples

The wheat samples were provided by the Martonvásár Crop Research Institute. They are not commercially available. Wheat samples were grouped by the Institute according to their infection levels.

To decrease the inhomogeneity by the single kernel measurements, wheat samples were grinded using a laboratory mill (sieve size 250 µm). Flour samples were then also measured with the same method as the wheat kernels. In the case of wheat samples 40 kernels were measured for each group. Flour samples were measured with 15 repeats. The numbers in the labels of the samples increase as the level of the *Fusarium* infection decreased. The samples with 1 in the labels were the most infected, MV4 samples were the least infected.

A separate group was also used for classifications: it was a not grouped sample of wheat (not investigated for *Fusarium* infection), it was labelled as V (group 1).

The wheat samples from the Martonvásár Institute were from 2010. The wheat sample "V" was from 2011.

Table 1: Sample labelling

Sample type	Labelling	Group classification
wheat	V	1
	MV1	3
	MV2	4
	MV3	5
	MV4	2
flour	V_F	1
	MV1_F	3
	MV2_F	4
	MV3_F	5
	MV4_F	2

2.2. Methods of measurement and data analysis

The spectra of each sample (wheat kernel and flour) were recorded on a MetriNIR 10-17 scanning-type spectrometer in the wavelength region of 700 to 1700 nm, with a spectral step of 2 nm (500 points per spectrum). The measurement was performed in transmission mode. 40 kernels were selected and measured from each wheat sample group. Flour samples were prepared by means of a laboratory mill. 15 spectra for each flour variety were recorded and used for statistical analysis.

For the evaluation of the recorded spectra there are many statistical multivariate methods to choose from. In this study the Polar Qualification System was used. Polar Qualification System (PQS) as an alternative qualification method was introduced by Kaffka and Gyarmati (1991) and was applied in several research approaches to distinguish sample groups (Gergely and Salgó, 2003, Seregély et al., 2004). According to PQS, a sample can be characterized by a centre of its polar spectrum. The centre, referred to as "quality point" can be defined by two data; in polar co-ordinate system it means an angle and a radius, or in the more usual rectangular Descartes co-ordinate system an abscissa and an ordinate (Kaffka & Gyarmati, 1998). It means a drastic data reduction. In case of a scanning spectrometer giving 700 spectral data, this reduction is 700–2. As the NIR spectrum is a fingerprint of the investigated material, PQS compresses the spectral data in a "quality point" on a two dimensional polar "quality plane" for qualification. The location of the quality point is influenced by all spectral data, thus, small differences in spectra may result separate clusters of quality points, resulting an effective method for discrimination.

3. RESULTS

The NIR spectra were recorded and using the 3 methods in the PQS system they were converted into quality points.

Discriminant analysis was used to classify the samples according to their quality points.

As an example the recorded spectra (average and deviations) of the sample MV4 is shown in figure 1.

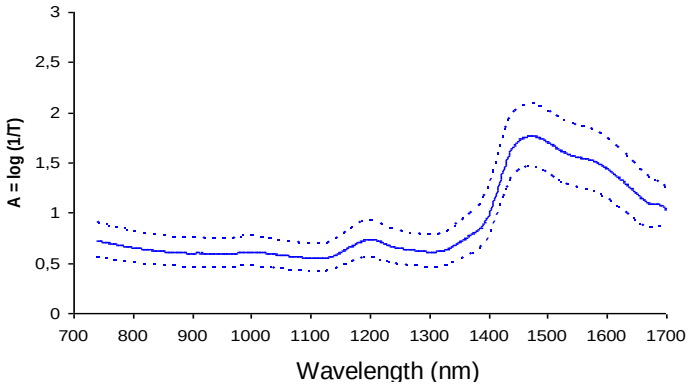

Figure 1: Average spectra (log (1/T)) and deviations of sample MV4

The quality points of the samples were calculated with the 3 different methods of the PQS (point, line and surface methods). An example of the results is shown in Figure 2.

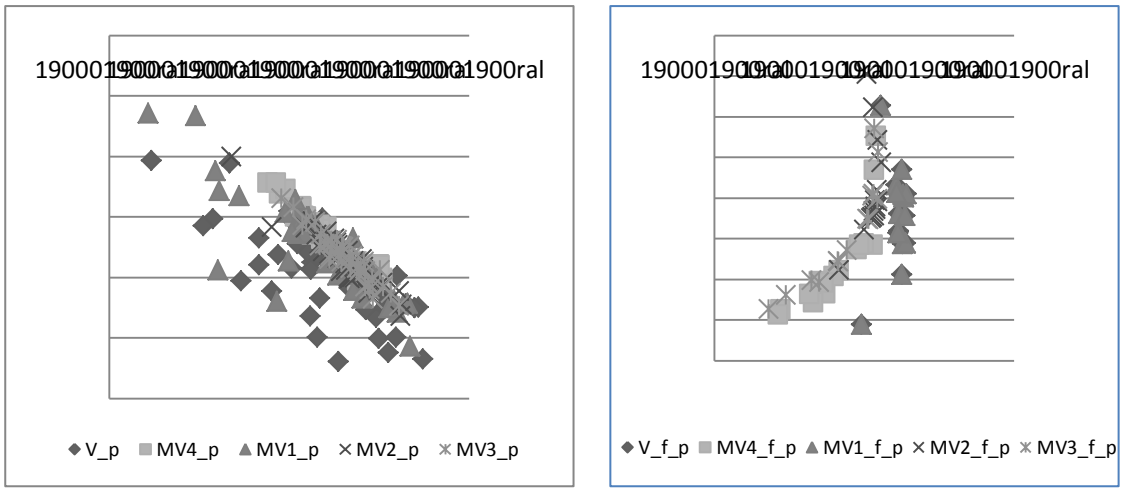

Figure 2: Quality points of the wheat (left) and flour (right) samples using PQS point method.

The results of the discriminant analysis showed an improved classification for the flour samples (compared to the original wheat kernel data). As an example in figure 3 the classification results are shown for the quality points of wheat (left) and flour (right) samples using PQS point method.

Classification Results[a,c]

		group	Predicted Group Membership 2	3	4	5	Total
Original	Count	2	22	2	5	11	40
		3	8	19	5	9	41
		4	12	9	7	12	40
		5	12	4	10	14	40
		Ungrouped cases	7	30	7	8	52
	%	2	55,0	5,0	12,5	27,5	100,0
		3	19,5	46,3	12,2	22,0	100,0
		4	30,0	22,5	17,5	30,0	100,0
		5	30,0	10,0	25,0	35,0	100,0
		Ungrouped cases	13,5	57,7	13,5	15,4	100,0
Cross-validated[b]	Count	2	22	2	5	11	40
		3	8	19	5	9	41
		4	12	10	5	13	40
		5	12	4	11	13	40
	%	2	55,0	5,0	12,5	27,5	100,0
		3	19,5	46,3	12,2	22,0	100,0
		4	30,0	25,0	12,5	32,5	100,0
		5	30,0	10,0	27,5	32,5	100,0

a. 38,5% of original grouped cases correctly classified.

b. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

c. 36,6% of cross-validated grouped cases correctly classified.

Classification Results[a,c]

		csopszám	Predicted Group Membership 2,00	3,00	4,00	5,00	Total
Original	Count	2,00	10	1	3	1	15
		3,00	0	14	1	0	15
		4,00	1	0	14	0	15
		5,00	7	0	7	0	14
		Ungrouped cases	0	14	1	0	15
	%	2,00	66,7	6,7	20,0	6,7	100,0
		3,00	,0	93,3	6,7	,0	100,0
		4,00	6,7	,0	93,3	,0	100,0
		5,00	50,0	,0	50,0	,0	100,0
		Ungrouped cases	,0	93,3	6,7	,0	100,0
Cross-validated[b]	Count	2,00	9	1	3	2	15
		3,00	0	14	1	0	15
		4,00	1	0	13	1	15
		5,00	7	0	7	0	14
	%	2,00	60,0	6,7	20,0	13,3	100,0
		3,00	,0	93,3	6,7	,0	100,0
		4,00	6,7	,0	86,7	6,7	100,0
		5,00	50,0	,0	50,0	,0	100,0

a. 64,4% of original grouped cases correctly classified.

b. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

c. 61,0% of cross-validated grouped cases correctly classified.

Figure 3: Discriminant analysis of the quality points of the wheat (left) and flour (right) samples calculated by the PQS point method.

However it was also showed, that using the full spectra the results have not corresponded fully with the grouping of the Martonvásár Institute.

Results of the statistical analysis are summarised in Table 2.

Table 2: Summary of the statistical analysis of the quality points

Sample groups	% of original grouped cases correctly classified	% of cross-validated grouped cases correctly classified
Wheat samples (point method)	38,5	36,6
Wheat samples (line method)	44,1	41,6
Wheat samples (surface method)	44,1	41,6
Flour samples (point method)	64,4	61
Flour samples (line method)	54,2	49,2
Flour samples (surface method)	54,2	49,2

4. CONCLUSIONS

The NIR is a useful tool for following the changes in the nutritional composition of the *Fusarium* infected wheat samples. The differences in the full spectra (700-1700 nm) of the investigated wheat sample groups were detected also in the quality points calculated by using PQS. However using the full spectra data the grouping with discriminant analysis not fully corresponded with the grouping done by the Martonvásár Institute. Grinding the wheat samples to flour resulted in better classifications (diminishing the inhomogeneity).

Our further aim is to narrow the spectra to the wavelength of the protein responses, and to repeat the calculations with these data.

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EFFECT OF CINNAMON AND THYME ESSENTIAL OILS AND THEIR MAJOR COMPONENTS ON THE FORMATION OF BACTERIAL AND YEAST BIOFILMS

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SUMMARY
Biofilms formed by bacteria and yeasts represent a continuous source of contamination in the food industry that may lead to food spoilage and food-borne diseases. These structures are more resistant to disinfectants than the planktonic forms and are difficult to remove from surfaces. Finding efficient natural antimicrobial agents to - at least partially - replace conventional food preservatives and disinfectants has gained more attention in the last decades. There seems to be a growing interest in using essential oils by the food industry as natural antimicrobial agents because of their excellent antimicrobial effect. In our study the anti-biofilm forming effect of cinnamon and thyme essential oils and their main components, cinnamon-aldehyde and thymol were tested against food spoilage microorganisms. Based on our results these oils and their components are good candidates for prevention of biofilm formation and could be used as disinfectants and/or food preservatives.

1. INTRODUCTION

Food contamination and food poisoning are big problems for health care therefore it is obvious that finding effective antimicrobial agents has become necessary. Biofilm formation is an extremely common phenomenon and most materials may rapidly become colonized by bacteria. Biofilms of both spoilage and pathogenic microflora are related to problems of food contamination, leading to lowered shelf-life of products and transmission of diseases (Chorianopoulos et al, 2008). The risk becomes even more serious since bacteria in biofilms have been shown to have higher resistance towards disinfectants compared to their planktonic counterparts (Gilbert et al, 2002; Høiby et al, 2010). Research for new substances in biofilm inhibition is therefore an important area of study. The recent negative consumer perception against artificial sanitizers has shifted this research effort towards the search of alternative, natural products that may at least partially replace synthetic substances (Burt, 2004).

Essential oils (EOs) obtained from plants have recently gained a great popularity and scientific interest. Previous studies have indicated that the EOs and extracts of edible and medicinal plants constitute a class of very potent natural antimicrobial agents (Burt, 2004). They act by damaging the cell wall and membrane, leading to cell lysis and leakage of cell contents (Burt, 2004). Thus these oils can help prevent the formation of biofilms and could be used in sanitization and food preservation processes.

In the present study we investigated the effect of cinnamon and thyme EOs and their major components (cinnamon-aldehyde and thymol) on the formation of bacterial and yeast biofilms.

2. MATERIALS AND METHODS

2.1. Microorganisms

Two Gram positive (Bacillus cereus var. mycoides, Bacillus subtilis), one Gram negative (Pseudomonas fluorescens) bacteria and the food spoilage yeast Pichia anomala were used to test the inhibition of biofilm formation. B. cereus, B. subtilis and P. anomala were incubated at 30 °C, and Ps. fluorescens at 25 °C.

2.2. Essential oils and components

Cinnamon and thyme essential oils were purchased from Aromax Natural Products Zrt. (Budapest, Hungary) and their main components, cinnamon-aldehyde and thymol were from Sigma-Aldrich (Hungary).

2.3. Determination of MIC values

Hundred µl of cell suspension (105 CFU/ml) was added to 96-well plates, followed by 100 µl medium containing the EO or component in appropriate concentrations. Positive controls contained the inoculated growth medium without any EOs or components and negative controls contained EOs or components in sterile medium. After 24 h incubation at corresponding temperatures, absorbance was measured at 600 nm. Decreases of the absorbance lower than 10% of the positive control samples were considered the MIC values. TWEEN 40 was added to the media for dissolving the oils.

2.4. Inhibition of biofilm formation and staining

Cell suspension (108 CFU/ml) was added to the 96-well plates, and after 4 h, the unattached cells were washed out. The EOs or components were added in MIC/2 concentration. After 24 h, wells were washed again, and cells were fixed with methanol and stained with crystal violet. Acetic acid was used for dissolving the dye and absorbance was measured at 590 nm.

3. RESULTS

All EOs and components showed anti-biofilm forming effect in various degrees. For B. cereus, cinnamon and cinnamon-aldehyde showed the best anti-biofilm effect and the treated cultures differed significantly from the control sample ($p<0.001$). Thyme and thymol had low inhibitory effect; the difference between the treated and untreated cultures was not significant (Fig. 1).

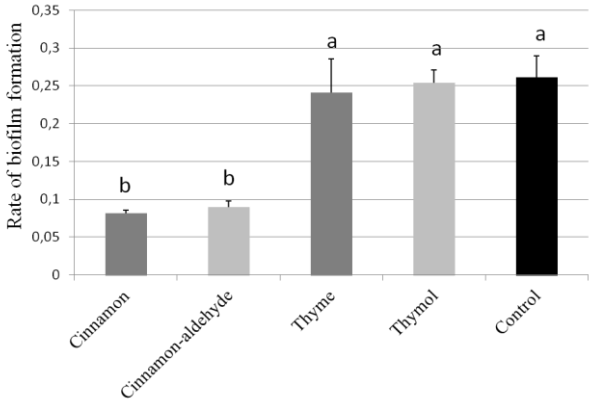

Figure 1: *B. cereus* biofilm formation after treatment with the investigated EOs and components. Different letters represent significant changes.

As well as *B. cereus*, *B. subtilis* resulted to be susceptible to treatment with cinnamon and its component ($p<0.001$). It showed higher susceptibility to thyme then *B. cereus* but thymol had no significant anti-biofilm forming effect in this case also (Fig. 2).

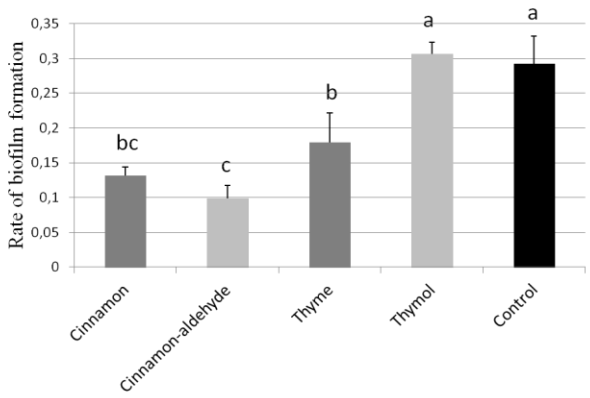

Figure 2: *B. subtilis* biofilm formation after treatment with the investigated EOs and components. Different letters represent significant changes.

Ps. fluorescens was less susceptible to cinnamon than to its major component, cinnamon-aldehyde. We obtained the opposite trend in case of thyme and thymol; thyme had a significant anti-biofilm forming effect but thymol showed no difference from the control sample (Fig. 3).

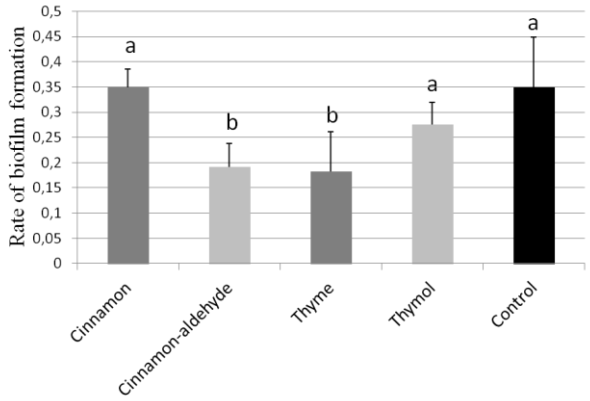

Figure 3: *Ps. fluorescens* biofilm formation after treatment with EOs and components. Different letters represent significant changes.

In case of *P. anomala* all treatments differed significantly from the control sample. The components resulted to be better inhibitors than the EOs and the best inhibitor was thymol ($p<0.001$) (Fig. 4).

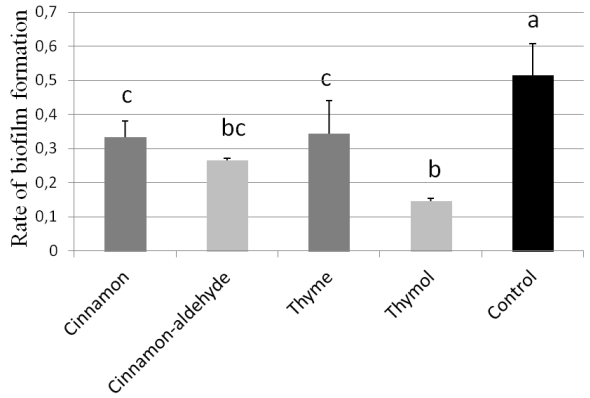

Figure 4: *P. anomala* biofilm formation after treatment with the investigated EOs and components. Different letters represent significant changes.

4. CONCLUSIONS

Gram positive and Gram negative bacterial biofilms showed different susceptibility to the investigated EOs and components. In all cases, cinnamon and cinnamon-aldehyde reduced the biofilm formation but thyme and thymol showed moderate or no effect on Gram positive bacterial biofilms. Further investigations are needed to elucidate the mechanism of action of biofilm formation inhibition. Food spoilage yeast P. anomala showed a high susceptibility to components, being more resistant to EOs. The EOs and components tested in this study are promising candidates to be used as disinfectants and/or food preservatives in the future.

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REGULATION OF FOOD SAFETY IN SERBIA

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SUMMARY

Serbia is about 10 years behind EU countries in the field of food safety laws and regulations.
The two main regulatory document is the Animal Health Law and the Food Safety Law. Due to legislative gaps implementation and verifying of HACCP system is shortcoming. In our work we analyzed the regulatory environment, and the implemented HACCP systems

EFFECT OF SOME FOOD COMPONENTS ON THE ANTIMICROBIAL EFFICACY OF SELECTED ESSENTIAL OILS

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SUMMARY

Most of the essential oils have excellent antimicrobial activity against food spoilage microorganisms and pathogens and are generally recognized as safe (GRAS). So, they have the potential to be used as natural preservatives in the food industry. The drawback is that, to achieve the same growth inhibition effect, usually a much higher concentration is needed in foodstuffs than in vitro in growth medium. Interactions between components of the food and the essential oil can lead to reduced or zero antimicrobial efficacy of essential oils. In our study the effect of vegetable oils, casein and soy protein concentrate, and BSA was investigated on the minimal inhibitory concentration (MIC) of juniper and marjoram essential oils. In most cases, independently of the concentration, addition of food components to the medium increased the MIC value, that is, decreased the antimicrobial effectiveness of the oil.

1. INTRODUCTION

Essential oils (EOs) have excellent antimicrobial activity against most of the food spoilage microorganisms and pathogens and are generally recognized as safe (GRAS). They have the potential to be used as natural preservatives in the food industry. The drawback is that, to achieve the same growth inhibition effect, usually a much higher concentration is needed in foodstuffs than in vitro in growth medium (Burt, 2004). Interaction between food and essential oil components can lead to diminished efficacy of the EOs. Essential oils have different mechanisms of action against microbes but the main target is apparently the cell membrane. They can cause loss of membrane integrity, leading to the release of cell contents and finally to cell death (Burt, 2004; Bakkali et al, 2008). To reach the cell membrane, a direct contact between EO components and the cell surface is needed. If EO components are solved in the fat phase of the food or are bound into the food matrix, they cannot reach the target microorganisms present in the aqueous phase of the food.

Most EO components are soluble in oils and fats. The saturation and the chain length of fatty acids in triglycerids influence the volatility of EO compounds.

The interaction between proteins and EO components can be reversible or irreversible. Reversible binding occurs via hydrogen bonds to aldehyde or alcoholic EO compounds or hydrophobic interactions with hydrocarbons while irreversible binding means covalent bonds to aldehydes. BSA and sodium caseinate was reported to bind volatile compounds mainly by hydrophobic interactions. Soy proteins bind the non-polar EO compounds by van der Waals forces and the more polar compounds (aldehydes and alcohols) by hydrogen bonds. Soy protein has a great potential for gel formation and the protein-protein interactions might decrease the number of potential EO component binding sites (Krisch et al, 2012).

In our study the effect of vegetable oils, casein and soy protein concentrate, and bovine serum albumin (BSA) was investigated on the minimal inhibitory concentration (MIC) of juniper and marjoram essential oils. The main component of marjoram EO is linalool, a terpene alcohol, and the main component of juniper EO is β-pinene, a bicyclic hydrocarbon; so all types of reversible bounds between proteins and EO components could be formed during our experiments.

2. MATERIALS AND METHODS

2.1. Strains and culture conditions

All the strains used were from the Szeged Microbiological Collection (SZMC; WDCM 987). The Gram positive (*Bacillus cereus var. mycoides* 0042) and Gram negative (*E. coli* 0582) bacteria were cultured on TGE medium (10g glucose , 5g pepton, 2.5g yeast extract for 1000 ml), or on LB medium (10g casein pepton, 10g NaCl, 5g yeast extract for 1000 ml) at 30 and 37^{o}C, while the yeast *Geotrichum candidum MB 102*, and the mold *Penicillium frequentans* were grown on malt extract medium (50 ml malt extract, 5g yeast extract, 5g glucose for 1000 ml) at room temperature.

2.2. Determination of MIC values

Determination of MIC values was done by the macro dilution method. Growth media were inoculated with 0.1 ml cell or spore suspension (10^6cfu/ml) and were supplemented with the investigated food components in the concentration range of 0-10%. All food components were purchased from local markets of Szeged. Marjoram or juniper essential oils stock solutions (100 µl/ml) were prepared in the growth media and were added in appropriate amount to the inoculated and supplemented media giving a final volume of 4 ml. After 24 h incubation at the above temperatures, the viable cell count was determined by the plate count method. MIC was determined as the concentration where no growth occurred.

3. RESULTS

3.1. Efficacy of essential oils in the presence of vegetable oils

Sunflower, olive and extra virgin olive oils were added to the media in 1, 5 and 10 % (v/v) concentrations. To aid the dispersion of oils, Tween 40 was used in 0.5%. As it can be seen in Figure 1 and 2, in almost all cases the addition of vegetable oils reduced the antimicrobial efficacy of the EOs by increasing the MIC values 2 to 4-fold. In most cases, except for *B. cereus* and sunflower oil, the diminishing effect was not dose dependent. This means that 2-4 times higher concentrations of EOs are needed in foods as preservatives when vegetable oils are present but there is no need to enhance the amount of EOs with increasing vegetable oil concentrations. Although the composition and the proportion of saturated and unsaturated fatty acids differs among the investigated vegetable oils it seems that it has no real impact on the MIC enhancing effect.

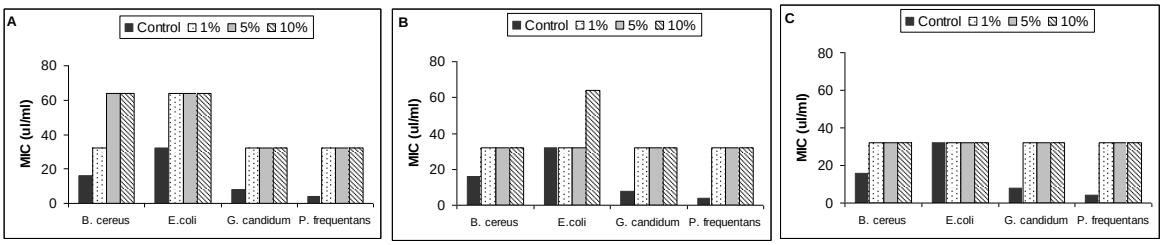

Figure 1: Effect of sunflower (A), olive (B) and extra virgin olive (C) vegetable oils on the antimicrobial efficacy of marjoram essential oil.

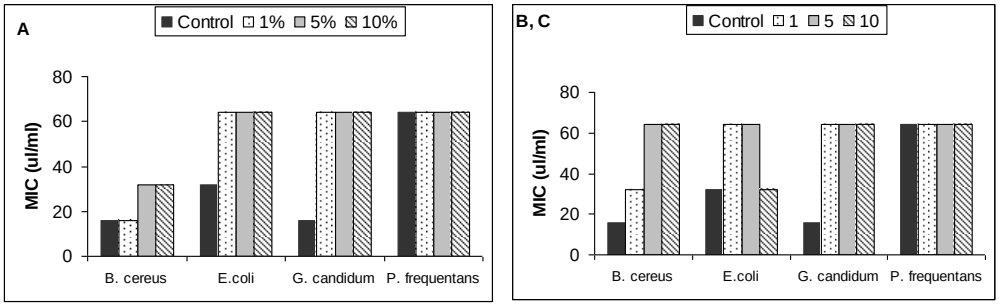

Figure 2: Effect of sunflower (A), olive (B) and extra virgin olive (C) vegetable oils on the antimicrobial efficacy of juniper essential oil.

3.2. Effect of milk proteins on the antimicrobial efficacy of EOs

In our study, BSA and casein concentrate (85%) was added in 0.5% (w/v) to the growth media to avoid protein-protein interactions. In all cases, MIC values were increased 4 to 6-fold after addition of the milk proteins. The MIC of marjoram EO was not influenced by the type of the added protein but the MIC of juniper EO on the investigated bacteria was higher in the presence of BSA compared to casein (Fig. 3).

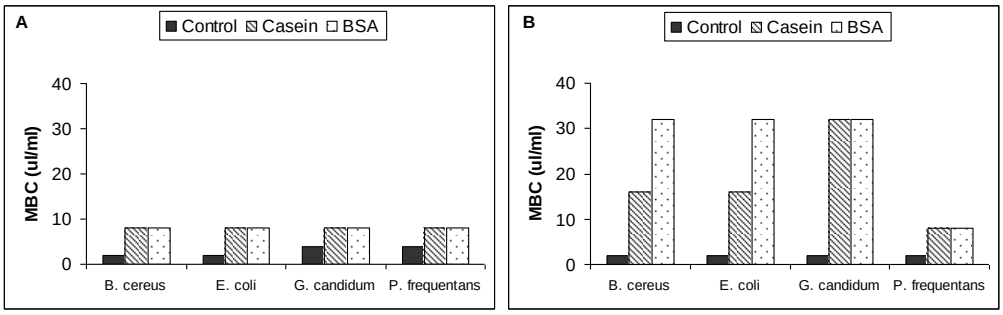

Figure 3: Effect of casein and BSA on the antimicrobial efficacy of marjoram (A) and juniper essential oil (B).

3.3. Effect of soy protein on the efficacy of EOs

Soy protein was added in the concentration range of 0.5 to 4 % (w/v). In low concentration soy protein increased the MIC of marjoram and juniper EO but the higher concentrations decreased the MIC to the original one or lower. The effect of soy protein was thus dose-dependent, and higher concentrations had no negative impact on the effectiveness of EOs (Table 1). In our previous study we observed a similar behavior for soy peptone (Tserennadmid et al, 2010).

Table 1: Effect of soy protein on the antibacterial efficacy of marjoram and juniper EO

Bacterium	Soy protein concentration (% w/v)				
	0 (Control)	0.5	1	2	4
MIC (µl/ml) of marjoram EO					
E. coli	32	64	32	64	32
B. cereus	16	64	16	8	4
MIC (µl/ml) of juniper EO					
E. coli	32	64	32	16	16
B. cereus	16	64	32	64	32

4. CONCLUSION

In all cases, addition of a food ingredient, oil or protein, to the culture medium diminished the antimicrobial capacity of the investigated essential oils. It seems that the main factors influencing this effect were the type of the essential oil (hydrophobicity of components) and the individual susceptibility of microbes to the investigated EOs. Different properties (saturation, fatty acid composition, protein structure) of the investigated food components within the same group, and the concentration of these components, had limited influence with the exception of soy protein. In higher concentrations the protein-protein interactions of soy protein led to a decrease in MIC values because of the saturation of binding sites. It seems very difficult to estimate the real antimicrobial activity of essential oils in foods. Foods have a complex matrix with the main ingredient groups of carbohydrates, fats, proteins and water, and all of these ingredients can interact with the EO components but also with each other, resulting in a dynamic binding-releasing behavior of the essential oil compounds.

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APPLICATION OF THE ELECTRONIC TONGUE TO DETECT COFFEE ADULTERATION WITH BARLEY

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SUMMARY

The current study presents an application of electronic tongue for the detection capability of the adulterant barley in brewed Robusta coffee. Robusta coffee samples were blended with roasted barley in a range of 1 to 80 wt%. Two commercial cereal coffee samples containing barley were included in the test series with a blending of 49 and 53 wt% of barley. A set of six sensors (STS, SWS, GPS, SRS, SPS, UMS) (originally seven) were chosen to employ the PCA for the data explanatory and subsequently to build a classification model by LDA. The different barley concentrations were classified correctly (100% calibration set, 96.7% for the validation set). To obtain an accurate model to predict barley in coffee, the sensor signals without any outliers were used to apply Partial Least Squares regression (PLS) successfully (R^2= 0.97, RMSEP= 4.99).

1. INTRODUCTION

Considering the coffee mainly two varieties are used for commercial purpose, known as coffee Robusta and coffee Arabica. The latter coffee species is mostly preferred due to improved organoleptic features and thus higher prices are obtained (Pizarro *et al.*, 2007). Beside the great difference in the final sale price depending on factors including coffee species (20-25%) and geographic origin, the vulnerability to adulteration with regard to substitutions of physical characteristics by several biological materials require thus assurance of quality (Pizarro *et al.*, 2007).

So far, new analytical approaches employing less selective sensors simultaneously, known as Electronic tongues (ET) or taste sensors can provide important information to extend the application of the device. Considering the authentication and thus especially the analysis of coffee as well as the usage of potentiometric sensors, most of the analysis are accomplished to classify different beverages (Legin *et al.*, 1997) or to obtain sensory profiles of coffee samples (Toko, 1998).

However, a lack of analysis to confirm authentication of coffee is observed using ISFET sensors such as ASTREE II-system (Alpha MOS, Toulouse, France) although the ability to identify and to discriminate the origin of mineral water was tested successfully (Sipos *et al.*, 2012). Hence, the objective of this investigation is to test the capability to detect fraudulent barley in coffee.

2. MATERIALS AND METHODS

2.1. Materials

Seven coffee samples were prepared as mixtures from 0 to 80 wt% of barley (B) in Robusta (R): **100R**, **99R1B**, **95R5B**, **90R10B**, **80R20B**, **50R50B**, **20R80B**. Two commercially available cereal coffee samples were also investigated with known Robusta – barley ratios: **Ot** (51R49B), **AF** (47R53B). All samples were originated from the same coffee factory.

2.2. Electronic tongue measurement

Coffee samples were analyzed by Alpha ASTREE II (Alpha MOS, Toulouse, France) potentiometric electronic tongue. The ET-system consists of an automatic sampler unit LS 16 containing 16 slots for samples. The used potentiometric sensors were based on ChemFET, more precisely ISFET chemical sensors. All measurements were performed with seven sensors (named SWS, BRS, GPS, SRS, STS, UMS and SPS according to the producer). The sensor array of this ET-system consists of an Ag/AgCl 3 M KCl reference electrode (Methrom AG).

Samples were gained by pouring 6 g of coffee with 100 ml distilled water. While conditioning by submersing each sensor in HCl was achieved, calibration was performed by measurement of a mixture of diluted **100R** and **20R80B** samples.

All measurements including both conditioning and calibration were performed at room temperature. Samples were analyzed for 120 s, whereat only stable potential were recorded. Each sample was measured nine times. Sensors were cleaned by rinsing with distilled water (for 15 s) between subsequent measurements, whereas distilled water was exchanged after each three cleaning stages.

2.3. Statistical analysis

The multivariate sensor data requires reduction, which was obtained by a non- supervised method (PCA). The originally high amount of variables (seven sensors) was summarized into a new set of uncorrelated variables, known as principal components (Edward R. and Selwayan, 2002). Furthermore, LDA was applied as a supervised method in order to evaluate the capability of discrimination of the current system maximizing the distances between classes by transformation of variables.

PLS regression (Edward R. and Selwayan, 2002) was used to obtain a quantitative model to provide the prediction of barley content by chemical sensor data. The validation was realized by leave-one-out (LOO) method All computations and chemometric analysis were carried out using the software R- studio 3.0 (Boston, USA).

3. RESULTS AND DISCUSSIONS

According to the preliminary data evaluation outlier's detection (four outliers for each sample) and sensors selection were performed. Based on showing less sensitiveness and discrimination ability five sensors (STS, SRS, SWS, SPS, UMS) were selected resulting in total number of 45 measurements.

3.1. Data exploration and pattern recognition

Figure 1 presents the PCA biplot, including both scores and loadings calculated on the data of different blends of adulterated coffee. PC1 (81%) and PC2 (15.6%) enable to visualize the information of five sensors in a two-dimensional space. In Figure 1 samples **20R80B**, **AF**, **OT** and **50R50B** are well separated. Furthermore, PC1 is a contributing factor which is related to the concentration (from left to right increasing concentration). However, PC1 and PC2 with five sensors show less discrimination capability for the barley concentration in the range 0 to 20 wt%.

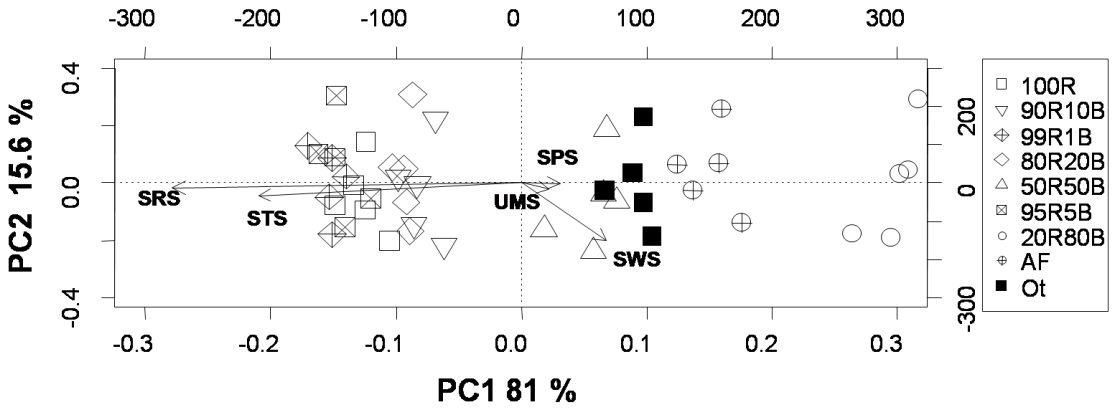

Figure 1: PCA biplot (scores and loadings) (PC1 and PC2) with the results of five sensors of the samples in the range 0 to 80 wt% barley

PCA accomplished as pattern recognition method only, displays weakness mainly in the low concentrated area. Hence, LDA is applied to classify adulteration.

3.2. Classification by Linear Discriminant Analysis

Classes for model building were defined according to barley adulteration. The first root (99.66%) separate all groups (Figure 2), but the distance between the classes **100R**, **99R1B** and **95R5B** as well as between **50R50B** and **OT** is not satisfactory.
The corresponding LDA-classification matrix (data not shown), which includes the calibration and the validation (LOO), only one misclassification in the calibration model is observed, i.e. 96.7% correct classification is obtained. Although a sample from **OT**-group (49 wt% barley) was assigned to **50R50B** (50 wt% barley), in terms of validation all samples were classified correctly (100%). In addition, the misclassification due to 1 wt% barley difference is acceptable but due to the small number of samples, classification for the **OT**-group amounts 80%.

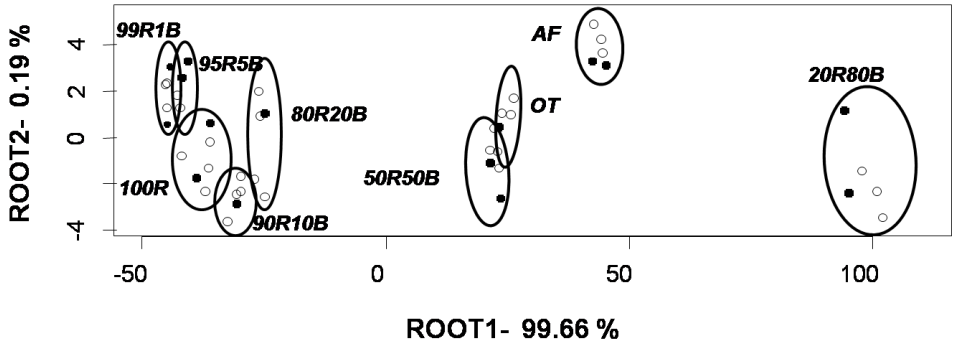

Figure 2: LDA- classification plot. Empty dots are contributed to calibration set; filled dots to the validation set

3.3. PLS regression model to predict the barley concentration

Multivariate calibration is applied for electronic tongue technique to predict the barley concentration in coffee. Based on cross-validation, four latent variables were selected. Validation was achieved due to leave out one-procedure. Figure 3 displays the calibration model finally selected as optimal with regard to predictive ability.

Despite a high correlation R^2 (0.97), the accuracy of prediction was not given for new values based on the sensor signals, especially for **100R** as well as for **80R20B**. According to

100R, all predicted values exceeded the measured value, whereas all values for **80R20B** were predicted below the measured. Thus no models were acquired with a low RMSEP. Concerning this model, a RMSEP of 4.99 features an accuracy of 3.99 wt% barley, so that no low level of barley in coffee can be predicted correctly.

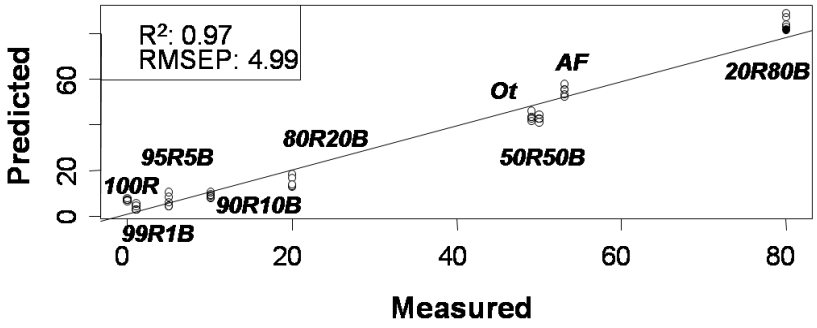

Figure 3: PLS- regression model to predict barley concentration in coffee blends

4. CONCLUSIONS

A good separation with regard to the high concentrated barley level indicates the capability as well as the possibility to detect adulteration in coffee. Based on chemometrics methods, a high classification power (96.6%) was obtained. Moreover, sensors SRS and STS display the highest discrimination ability, but overlapping of the different samples of the same group is an indication of less sensitivity particular for the low concentrated level (Lvova *et al.*, 2003). The extension of the given sensor array consisting of similar ISFET- sensors to SRS and STS, could increase the resolution. According to the prediction power of the PLS model, the desired accuracy is not given. Nevertheless, electronic tongue systems seem to be a promising procedure to be considered in future applications to quantify different adulterants in coffee and to gain further information, e.g. organoleptic characteristics.

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PRODUCTION OF PROBIOTIC ICE CREAM BASED OF LACTOSE HYDROLYSED MILK, SUPPLEMENTED WITH BLACK CURRANT

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SUMMARY

The viable cell count of a new type of probiotic ice cream - based on lactose-hydrolysed milk, supplemented with black currant jam - was examined. It is well known, that black currant is rich in antioxidants and vitamin C .This new type of ice cream was supplemented with 10% of probiotic yoghurt and 10% of black currant jam.The probiotic yoghurt was based on lactose hydrolysed milk which was inoculated with 5% of Bifidobacterium bifidum and Lactobacillus casei ssp.rhamnosus and was fermented at 45°C, until the complete coagulation. The acidity (SH°) and the probiotic cell count (N) by Breed method were determined during the yoghurt fermentation.The viable cell count of the ice cream (N) was controlled in modified MRS agar after mixing and after deep-freezing of the ice cream for 2 months.The organoleptical characters of the probiotic ice cream were qualified by 10 persons after 2 month storage.

1. INTRODUCTION

Recently, a lot of attention is paid to the health promoted properties of certain lactic acid bacterium strains, some of which have been promoted as probiotics. Probiotics are viable cell preparations or food containing living bacteria that after consumption of a certain number have beneficial effects on the health of the host. Examples are lactobacilli and bifidobacteria that are applied in yoghurts and fermented milks, or that are supplied as pharmaceutical preparations. (W. Pfanhauser, 1996).

Probiotic lactic acid bacteria contribute to the balancing of a normal, healthy intestinal microbiota, and to the prevention and curring of the diaorrhea, and may protect against gastrointestinal infections by pathogenic strainns, stimulate the immunity sistem, display anti-carcinogenic activities,etc.Howewer , only a very limited number of these „health claims" has been approved scientifically.(Luc the Vuist, 2003) .

Prebiotics are non-digestibile food ingradients that affects the host beneficially by selectively stimulating the growth and the metabolic activity of a limited number of naturally present or orally introduced bacteria in the colon, particularly bifidobacteria, and dereby improve host health. (W. Kneifel, 2002).

Dietary fibre content is reduced considerably by milling processes. In former times it was assumed to be useless for human consumption but only for feeding purposes applicable. Now it has turned out that fibres contain valuable amounts of trace elements and minerals as well as vitamins.But beside these dietary fibers have additional health effects (Bíró G., 2000).

There are fruits also, which have lot of useful components. For example the black currant (Ribes nigrum) is rich in different components, as potassium, phosphorous, iron and flavonoids, panthotenic acid, vitamin C and E, calcium, magnesium, zink, unsaturated fatty acids (alpha linolenic acid and gamma – linolenic acid), anthocyanins. Poliphenol content is very important, too. Medicinal use: black currant have antimicrobial, anti-cancer, antioxidant and diuretic properties.Berries are said to be effective in cases of rheumatism, gout , arthritis. Due to its high content in vitamin C black current is very often used in treatment of diseases related to cardiovascular system. (P.C.H.Hollman, M.G.L. Hertog,. M.B.Katan, 1996)

Production of new type ice cream, based on special milk, supplemented with probiotic yoghurt and black currant jam may give a functional sweetmeet for lactose sensitive people.

Xylitol,which is a populare sweetener, may be useful for diet of the lactose sensitive people.Nowadays this compound is often applied in the reform dietetic foods as prebiotic.

2. MATERIALS AND METHODS

2.1. Magic milk , Naszálytej Corp. (protein: 3,3g/100g, fat: 2,2g/100g , carbohydrate:4,5g/100g , lactose 0,1g/100g , mineral salts: 0,6g/ 100g)

2.2. Sweet cream (Friesland Foods Professional, Belgium, Lummen): protein: 2g/100g), carbohydrat: 11g/100g, total vegetable oil: 20g /100g (dehydrogenated : 5g/100g)

2.3. Black currant jam , Sirenex Corp., Bulgaria (45g fruit/100g , 17g glucose-fructose syrup/100g)

2.4.P robiotic starters : Lyophilised cultura of Bifidobacterium bifidum and Lactbacillus casei ssp. rhamnosus were resuscited and precultured for the inoculation of the probiotic yoghurt fermentation in lactose hydrolysed milk, at 37°C/ 12 hours.

2.5. Yoghurt fermentation

The temperature of 600ml lactose - hydrolysed milk was stabilisied at 45°C and after it this milk was inoculated with 15ml culture of Bifidobacterium bifidum and 15ml culture of Lactobacillus casei ssp. rhamnosus. The fermentation was run until the complete coagulation. During the fermentation the acidity was measured with Soxlet-Henkel method , as SH°.Breed method was applied to obtain the cell number of Bifidobacterium bifidum and Lactobacillus casei ssp. rhamnosus .

2.6. Xylitol , Nyírfacukor Kft., Budapest, www.nyifacukor.hu

2.7. Orange juice (Cappy Pulpy, Coca-Cola HBC Magyarország Kft., Dunaharaszti) carbohydrat: 10,6g/100ml , fat: 0g/100ml , dietary fiber: 0,6g/100ml , Na: 0,6g/100ml

2.8. Production of ice cream

- 100g xylit and 100 ml probiotic yoghurt was mixed with 800ml whipped cream.
- 300 ml was separated from this mixture , and 100ml orange juice was added to this probiotic cream. (A sample)
- The 700ml probiotic mixture was divided into two part .

360ml of this probiotic cream was supplemented with 40g black current jam. (B sample)

- 340g of black currant jam was added to 340 ml of the probiotic whipped cream base. After it was mixed and divided into two part and 400ml was separated from this mixture (C sample). The residual 28ö ml of this probiotic mixture was supplemented with 120 ml orange juice (D sample)
- The samples were refrigerated in ice-cream batch-freezer.

2.9. MRS-agar was applied to control the viable cell count of the ice creams after freezing and after storage, for 2 months.

3. RESULTS

3.1. The values of acidity and probiotic cell number during the fermentation

The Table 1. demonstrated for us the increases of SH° and lg probiotic cell number (lgN).

Table 1: The SH° and lg N of probiotic bacteria at the time of fermentation

Time (min)	SH°	lgN (Bif. Bifidum)	lgN (L.rhamn.)
0	12,24	6,81	6,05
60	19,08	7,47	6,44
120	24,12	8,17	6,83
180	29,52	8,48	7,1
240	33,12	8,35	7,4
300	35,28	8,31	7,27

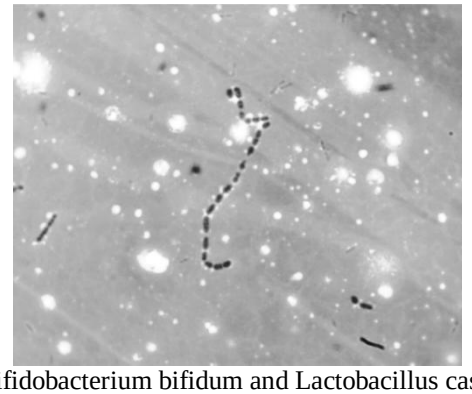

Figure 1: **Breed stain of** Bifidobacterium bifidum and Lactobacillus casei ssp. rhamnosus (1500X)

3.2. The sensory evaluation of different probiotic ice-creams (Kramer method)

Table 2: Ranking places of different probiotic ice-cream samples

Panellists	Runking places of different ice-cream samples			
	A sample	B sample	C sample	D sample
1. panellist	2. place	3. place	4. place	1. place
2. panellist	3. place	1. place	2. place	4. place
3. panellist	3. place	1. place	4. place	2. place
4. panellist	1. place	2. place	3. place	4. place
5. panellist	3. place	2. place	1. place	4. place
6. panellist	1. place	3. place	2. place	4. place
7. panellist	2. place	1. place	4. place	3. place
8. panellist	2. place	1. place	3. place	4. place
9. panellist	3. place	1. place	2. place	4. place
10. panellist	3. place	1. place	2. place	4. place
Runk Sums:	23	16**	27	34##

Symbols: ## : Runk sum more than 33: the sample worse than the other samples (SD= 99%)
** : Runk sum less than 17: the best sample (SD=99%)
Runk sums : 17 – 33: no significant difference at the 99% level of significance

3.3. The viable cell count (N) of probiotic bacteria in the ice cream samples

Table 3: The values of lg viable cell count (lgN) in the ice cream samples at the time of frozen storage

Time (month)	lgN			
	A sample	B sample	C sample	D sample
0	7,6	7,7	7,5	7,8
1	7,4	7,5	7,2	7,4
2	7,0	7,3	7,1	7,2

4. DISCUSSION

Suitable acidity (SH° = 35) was resulted by probiotic bacteria, by Bifidobacterium bifidum and Lactobacillus paracasei ssp. in the yoghurt, based on lactose hydrolised milk, after 5 hours. At the end of fermentation the cell number was more than 100million /ml in case of Bifidobacterium bifidum and 10million/ ml in case of Lactobacillus casei ssp. rhamnosus.(Table 1.)

The microscopic photo (fig.1.) of Breed stain (Table 1.) demonstrated, that number of Bifidobacterium bifidum was about tenfold higher than number of Lactobacillus casei ssp. rhamnosus in the yoghurt. To mix 10% of this yoghurt into the ice-cream may be a succesful to get a new probiotic product for the lactose sensitive people.

The new types probiotic ice-creames which were supplemented with orange juice or black currant jam and orange juice + black currant jam together, were tasted by 10 panelists. There was significant difference betwen the samples: the supplementation with 10% black currant jam was the best.(Table 2.)

The viable cell count of probiotic bacteria was appropriete in all samples after storage. In all cases it seemed to be higher than 10 million probiotic cell /ml of ice-cream. (Table 3.)

Usually the requirement of probiotic cell count is at least 1 million cell / g or cell / ml in the product.

The new types probiotic ice creams give a good possibility for the lactose sensitive people to get a probiotic sweetmeet. The supplementation with 10% black currant jam resulted the most tasty probiotic ice-cream sample, which was rich in vitamin C and antioxidants , as well as antocyan .

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DEVELOPMENT OF AGE-SPECIFIC GASTROINTESTINAL MODEL SYSTEMS

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There are several age-related changes in the human gastrointestinal tract, which express through altered enzyme compositions of the digestive juices and changes in the constitution of the intestinal microbiome. Based on these differences, our aim was to develop an *in vitro* gastrointestinal model of infants and elderly.

Our basic gastrointestinal model simulates the digestion process in adults in a simplified manner by applying physiologically based conditions, i.e. chemical composition of digestive juices, pH and residence time period typical for each compartment. The model describes a four step procedure simulating digestive process in mouth, stomach, small intestine and large intestine. The temperature of the model was set to 37 °C. The incubation time of the different compartment was five minutes in mouth, two hours both in stomach and small intestine and 48 hours in the large intestine. In the large intestine we applied the six most common bacterial species of the gut, two probiotic (*Bifidobacterium*, *Lactobacillus*), and four (*Escherichia*, *Enterococcus*, *Clostridium*, *Bacteroides*) other strains. Infant's digestive system is still very immature. The stomach is small, the gastric emptying is slower, which results longer incubation time in the stomach compartment *in vitro*. Infant's produce much lower level of digestive enzymes, because of the insufficient developed pancreas. Thus, in our infant model we reduced the level of pancreatic juice. *Bifidobacterium* and *Lactobacillus* species were used in the large intestinal compartment. The characteristics of elderly digestion are reduced saliva production in mouth and reduced intensity of chewing which results in our model smaller amount of α-amilase, and reduced mechanical stress. Hence, larger food parts enter the stomach, where reduced gastric juice production is present, which contained reduced level of HCl and pepsin and longer incubation time in this compartment. According to the muscle atrophy in the large intestine, longer transit time is applicable. Age-related changes in the elderly microbial community are increased number of facultative anaerobes, in conjunction with a decrease of beneficial organisms such as the anaerobic bifidobacteria, and general reduction in species diversity.

The investigated functional components showed altered digestibility in the age-specific gastrointestinal models and resulted different microbiological changes.

Through the modelling of the gastrointestinal tract of infants and elderly, we provide a useful alternative instead of the human clinical trial in these sensitive ages.