New insights into the acidification techniques of fruit mash during fermentation process

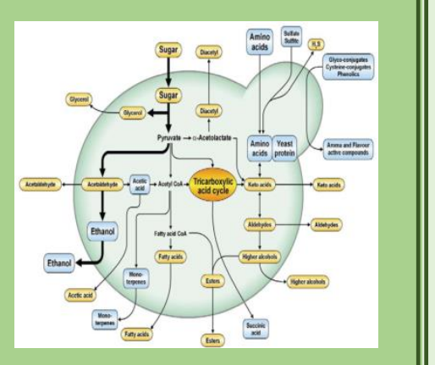
Fatjona Fejzullahu, Rajmund Ujszászi, Olívia Csernus, Szilárd Kun

Hungarian University of Agriculture and Life Sciences, Institute of Food Science and Technology,
Department of Bioengineering and Alcoholic Drink Technology, Budapest, Hungary.

INTRODUCTION

The production of alcoholic beverages, especially alcoholic fermentation, is accompanied by a number of concerns, such as spoilage and undesirable changes in flavour associated with the metabolic activity of undesirable microorganisms (Jeon et al., 2015). It is well known that lower acidity and higher pH generally support the growth of microorganisms, including several unwanted or spoilage species. Therefore, it is essential to monitor the pH and acidity of the medium throughout the fermentation. The most common practice of acid management worldwide includes the addition of organic acids at the start of alcoholic fermentation in order to prevent the proliferation of spoilage microorganisms (LAB and other bacteria, molds, and foreign yeasts). Nowadays, as both science and industry are open to innovation, new alternatives that offer acid protection of the fermentation medium are being researched. Attention is being paid to specific microorganisms that can offer a natural acidification and rapid pH drop by producing mostly lactic acid and other organic acids as part of their metabolism (Vilela, 2018; Morata et al., 2018; Vicente et al., 2021).
The goal of this study was to test the efficiency of different chemical and microbial acidification techniques in the process of fermenting fruit mash and their impact on the quality of the final spirit.

MATERIAL AND METHODS



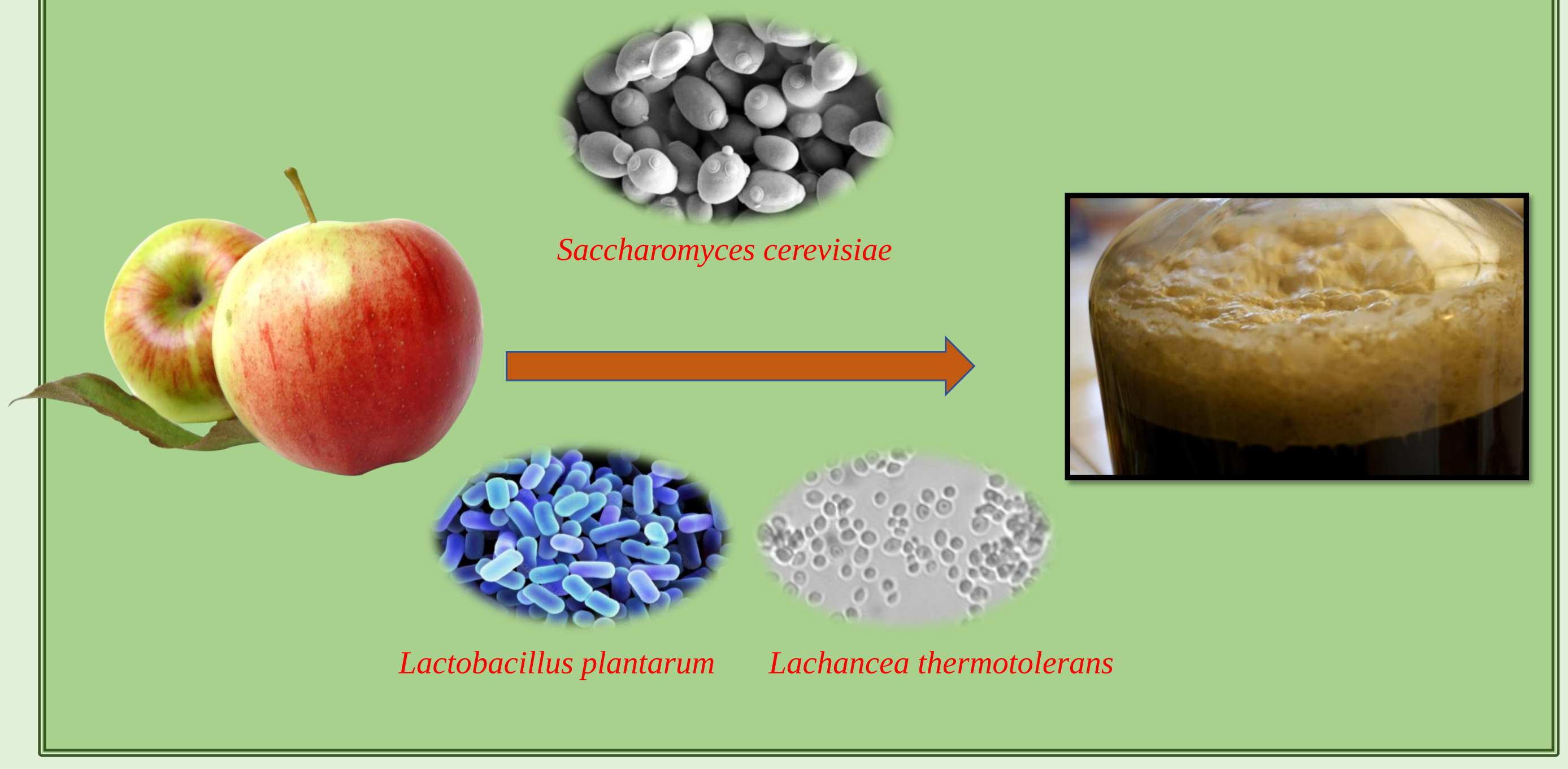
Raw material: Idared Apples
Starter culture:
- ✓ Uvaferm 228 (*Saccharomyces cerevisiae,* Lallemand)

Mashing and Fermentation conditions:
- ✓ Pectin degradation using Lallzyme^{TM} HC enzyme
- ✓ Nutrients (Uvavital^{TM} complex nutrients)
- ✓ Acidification:
  1. 25% phosphoric acid
  2. 25% phosphoric and 25% lactic acid in a ratio of 90:10
  3. 25% phosphoric and 25% lactic acid in a ratio of 80:20
  4. 25% phosphoric and 25% lactic acid in a ratio of 70:30
  5. 25% phosphoric and 25% lactic acid in a ratio of 60:40
  6. Harvest LB-1 (*Lactobacillus plantarum*, Chr. Hansen)
  7. Laktia^{TM} (*Lanchancea thermotolerans*, Lallemand)
  8. WildBrew^{TM} Sour Pitch (*Lactobacillus plantarum*, Lallemand)

- ✓ The fermentation was carried out at 18±1°C for two weeks.

Distillation: Laboratory glass-distillation equipment

Analytical methods: Fermentation process was followed with traditional and modern analytical methods:
- ✓ Dry matter content - Digital refractometer
- ✓ Reducing sugars content - Schoorl-Regenbogen method
- ✓ Total titratable acidity - Potentiometric method
- ✓ Sugars and organic acid concentrations - HPLC
- ✓ Ethanol content - Automatic density meter
- ✓ Ester content - Back titration
- ✓ Volatile organic compounds in the distillate - GC-FID



RESULTS

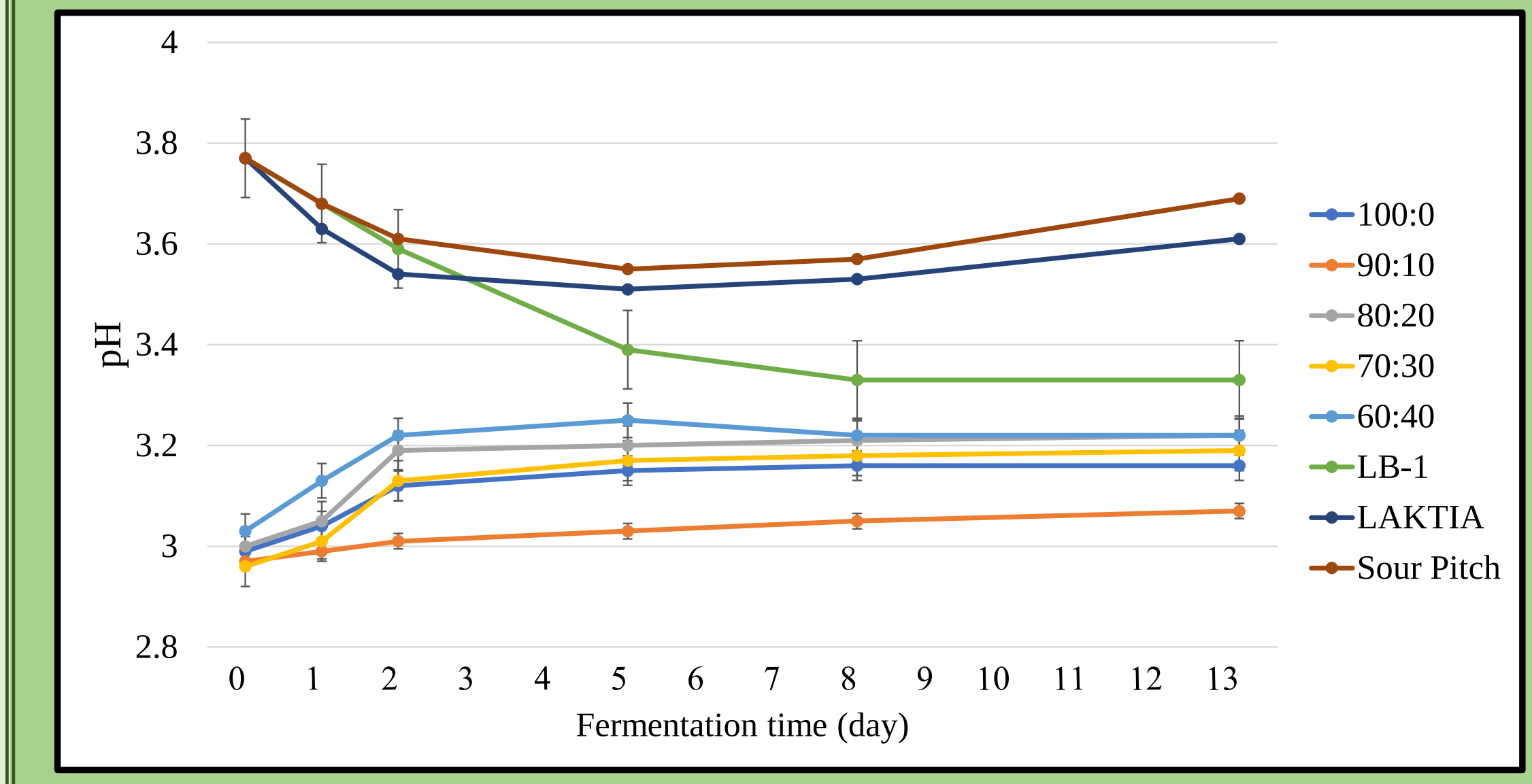


Figure 1. Changes of the pH during fermentation process

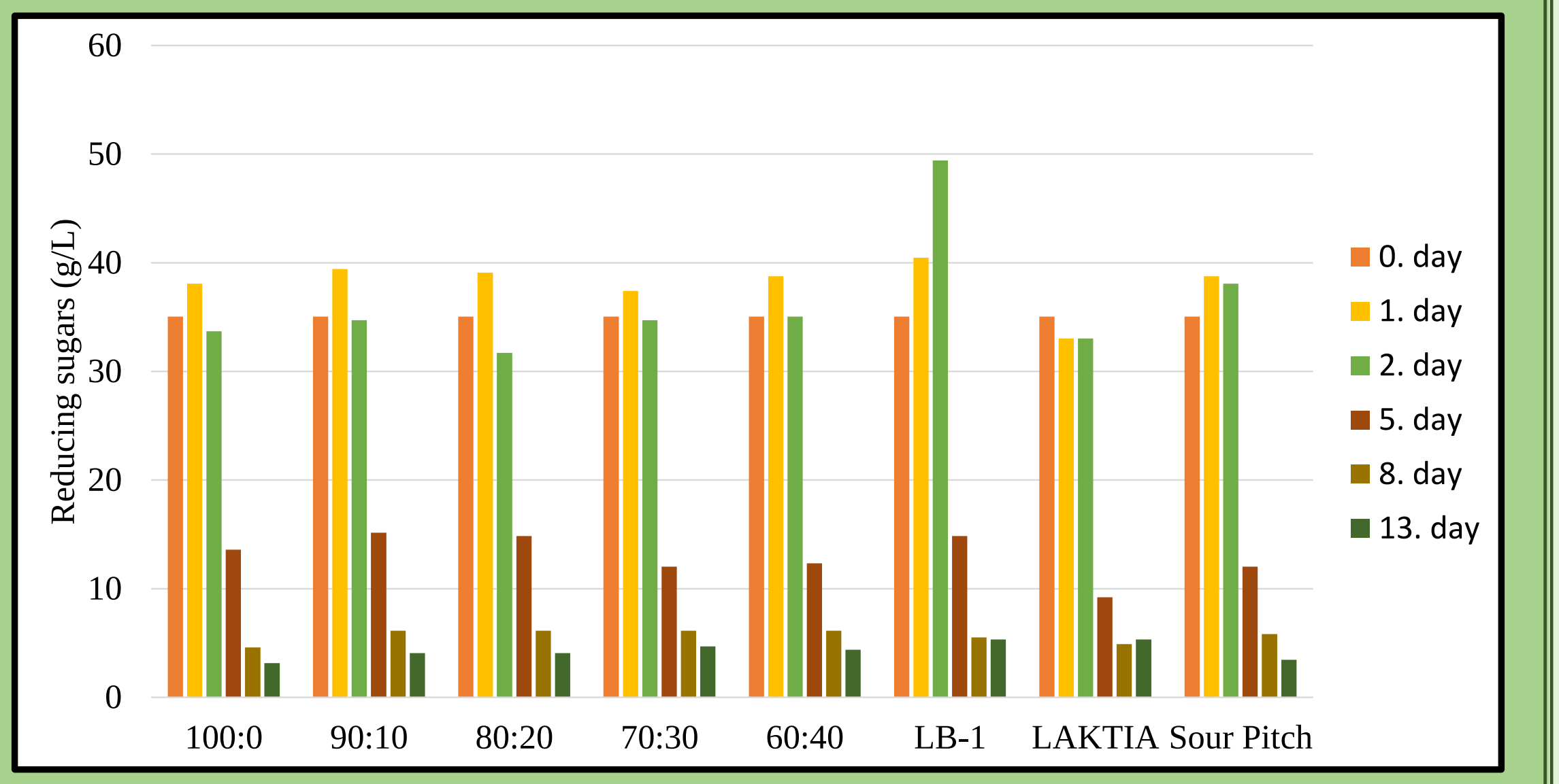


Figure 2. Changes of the concentration of reducing sugars during fermentation process

- ✓ In the case of acidified samples, a sudden increase in pH is seen after the onset of fermentation. This may be due to the fact that the fermentation has entered an anaerobic stage, alcohol is being produced through the metabolic activity of yeasts, and as a result, the solubility of the salts has changed, which has caused a pH-increasing effect. However, every time the pH fell in the range of 2.9-3.3, so that the growth of possible harmful microorganisms and thus their metabolic products, which degrade the quality of the distillate, remained inhibited throughout. Bioregulators were able to reduce the pH of the fermentation medium by a certain amount. We can highlight the strain *L. thermotolerans*, which was able to reduce the pH of the medium to 3.33 (Figure 1).

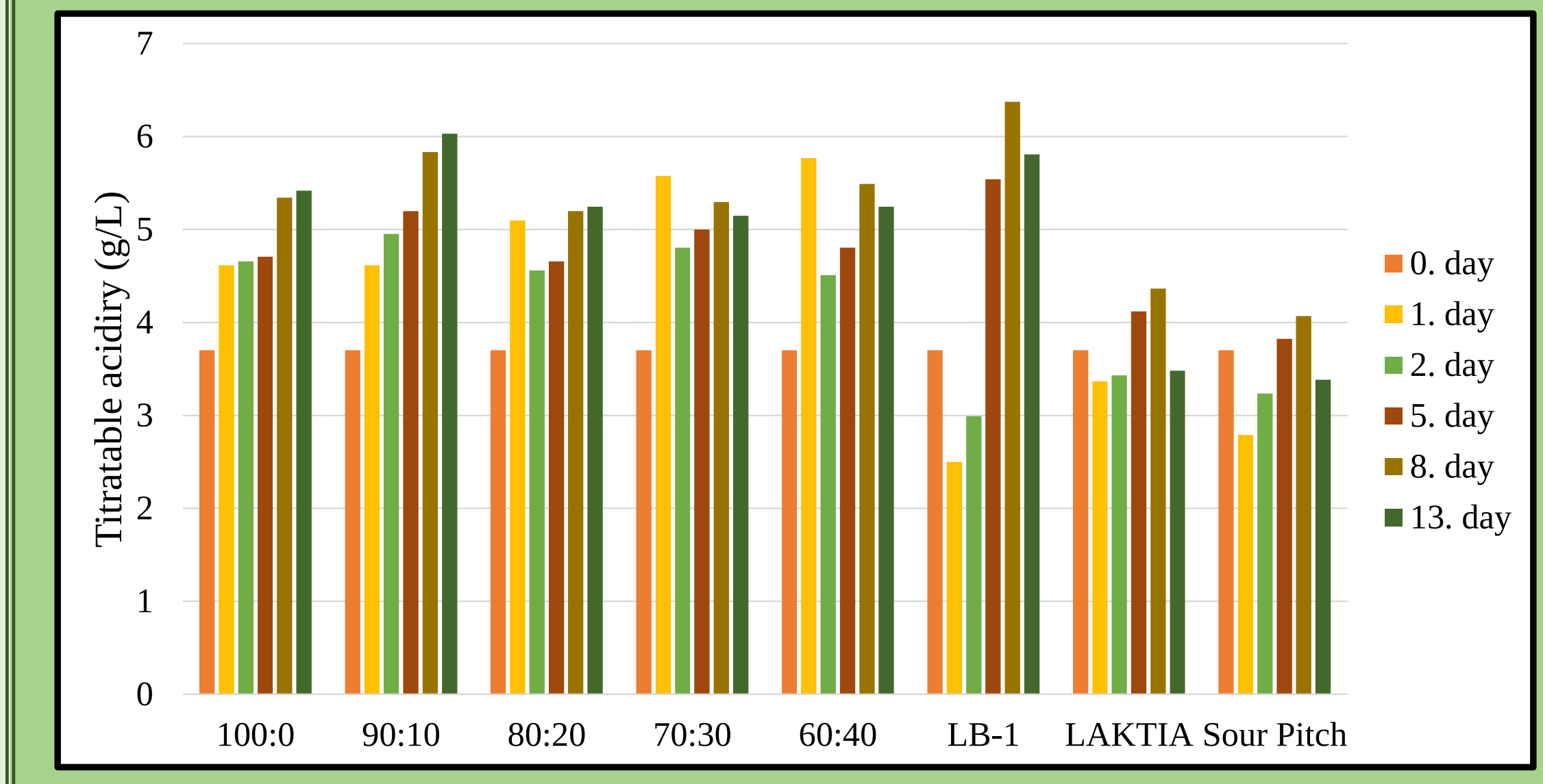


Figure 3. Changes of the acidity during fermentation process

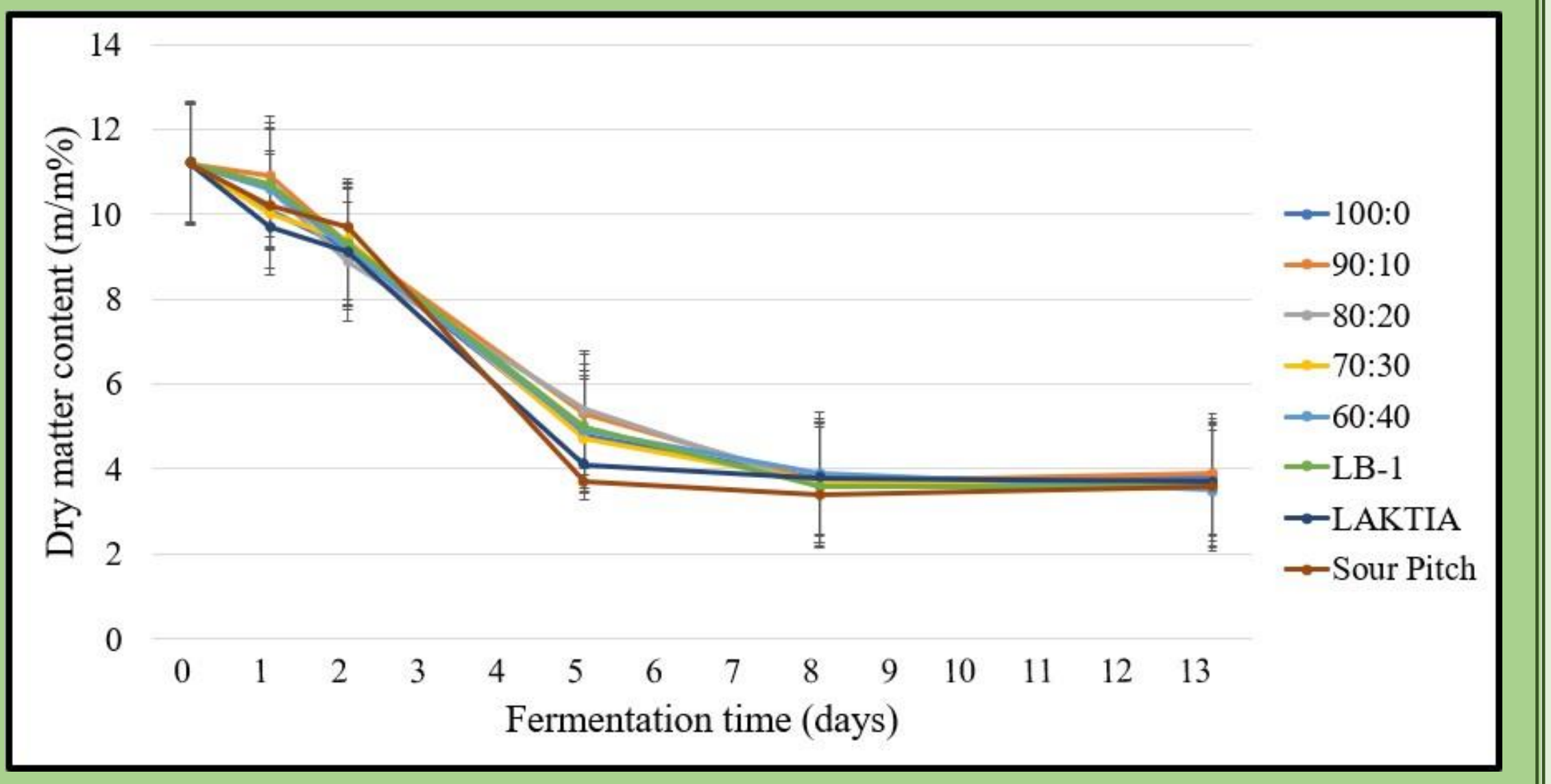


Figure 4. The profile of refraction during fermentation process

- ✓ In each fermentation medium, the yeast gradually utilizes the available carbohydrate source. At the beginning, a very strong carbohydrate breakdown is observed. Regarding the dynamics of refraction change, the bioregulators, Lactia and Sour Pitch, showed outstanding behaviour (Figure 2 and 4).
- ✓ The titratable acid contents indicate that the LB-1 (*Lactobacillus plantarum*) performed more intensive acid production throughout the fermentation process (Figure 3).
- ✓ Among all the samples, the greatest alcohol content was produced in the sample acidified by only phosphoric acid (5.5 V/V%). Whereas the lowest alcohol content resulted from the mash inoculated with *L. thermotolerans* (Laktia). The volatile acid content of the fermented mash ranged from 0.24-0.38 g/l, all below the critical limit of 0.5 g/L (Table 1).

	Volatile acidity (g/L)	Alcohol content (V/V%)	Ester content (mg/100ml a.a.)
100:0	0.31	5.5	160.4
90:10	0.25	5.3	346.2
80:20	0.31	5.3	171.4
70:30	0.24	5.3	237.3
60:40	0.31	5.3	224.5
LB-1	0.26	5.1	242.3
Laktia	0.31	4.9	258.7
Sour-Pitch	0.38	5.3	320.5

Table 1. Volatile acids and alcohol content of fermented mash, and ester content of the final distillate

- ✓ HPLC measurements revealed no major differences in sugar concentrations between samples throughout the fermentation process. In the samples supplemented with phosphoric and lactic acid in the ration of 60:40, the yeast was able to utilize glucose, fructose, and sucrose significantly better (Figure 5).
- ✓ The acidity contributes significantly to the sensory perception and quality of Pálinka. Some organic acids originate from the fruit, others form during alcoholic fermentation. The most acetic and lactic acid was produced by the microorganisms LB-1 and Laktia (Figure 6).
- ✓ The identified volatile compounds which contribute to the aroma and flavour of the final distillate include mainly higher alcohols and esters. Higher alcohols present in greater amounts in all samples included 2-methyl-1-butanol, isoamyl alcohol, 1-propanol, 2-butanol, and 1-butanol. The predominant ester in all samples was ethyl acetate, with the highest concentration in the sample 70:30 and the lowest in 90:10. In the samples inoculated with bioregulators, moderate levels of ethyl acetate were produced. In the samples named Laktia, LB-1 and Sour-Pitch, trace amounts of isoamyl acetate were detected. A number of other esters were detected in different samples, such as isobutyl acetate, 2-phenethyl acetate, diethyl succinate, ethyl benzoate, and ethyl-L-lactate (Figure 7).

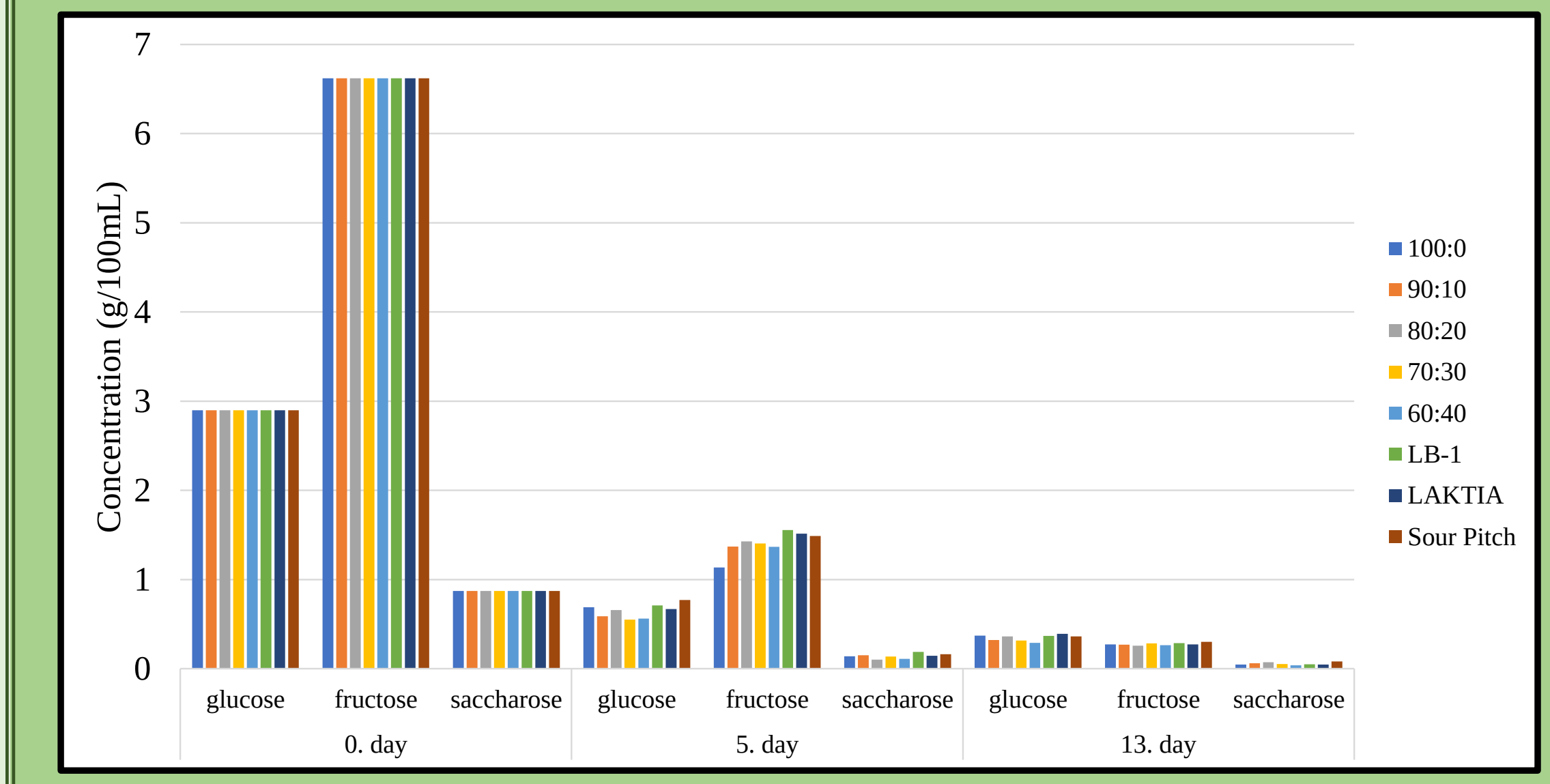


Figure 5. Changes of carbohydrates concentrations during fermentation

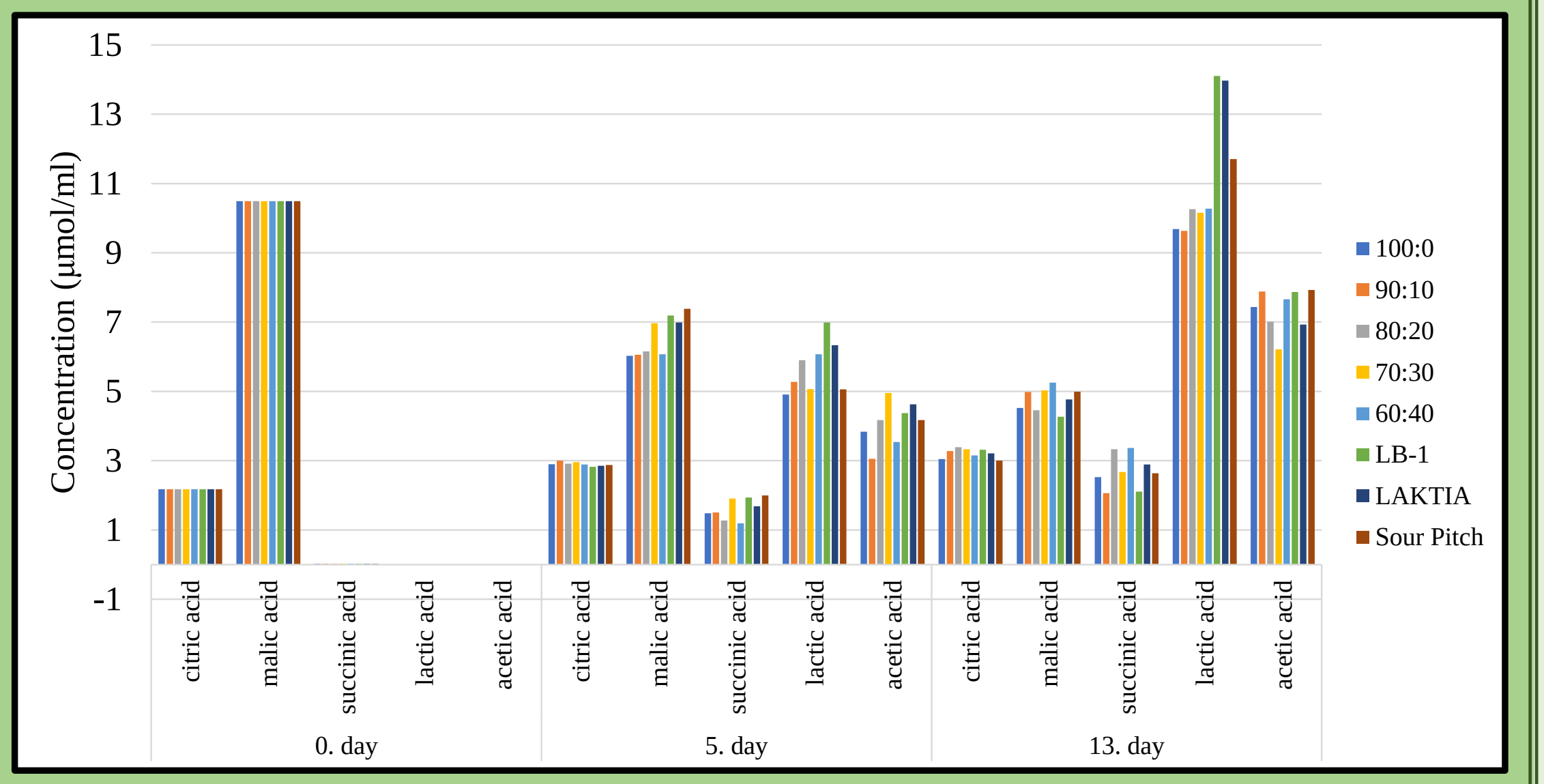


Figure 6. Changes of the organic acids concentrations during fermentation

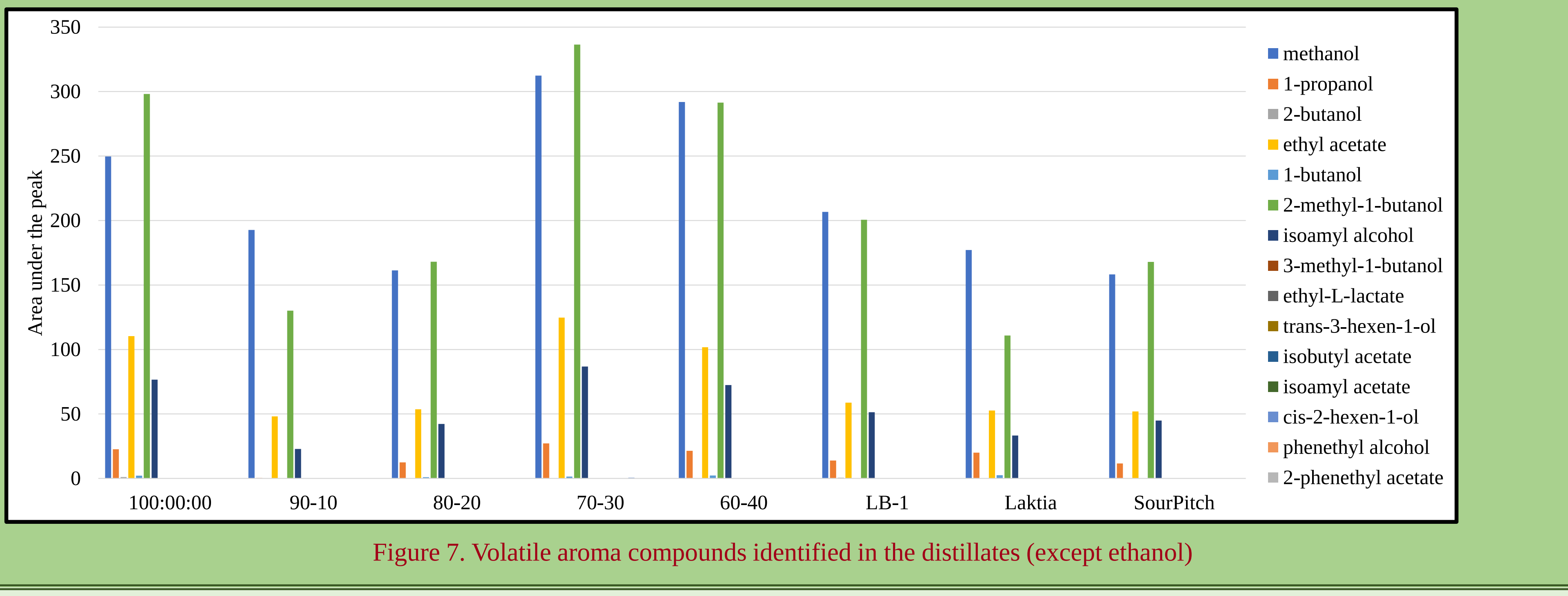


Figure 7. Volatile aroma compounds identified in the distillates (except ethanol)

CONCLUSION

- ✓ Improvements in mash acidification techniques used for fruit spirit production are of the utmost importance, as they are directly linked to spirits' improved organoleptic quality. As per traditional acidification using organic acids, results showed that different ratios affect yeasts' metabolism differently, and thus the metabolites produced.
- ✓ The outcomes of our experiment support the possibility of using *Lactobacillus plantarum* and *Lachancea thermotolerans* for acidification, providing a potential solution for fruit mashes with high pH and high sugar concentration. However, it is clear that more research is needed before it can be used as a starter culture for biological acidification in alcoholic beverage production.

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