Optimization of ultrasound-assisted extraction of antioxidant compounds from Hawthorn using response surface methodology

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MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM
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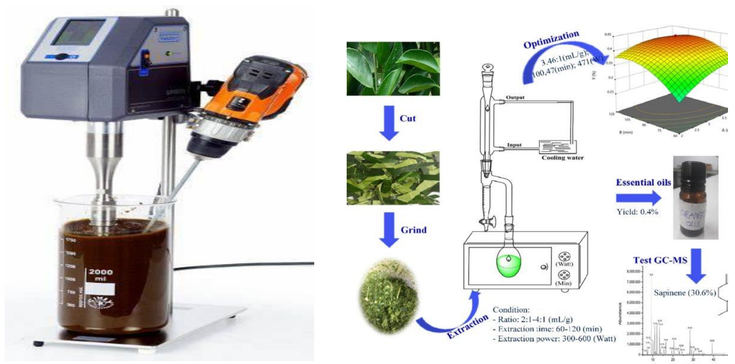
INTRODUCTION:

- Hawthorn is a common name for all plant species in the genus Crataegus, that is belonging to the Rosaceae family *(Dahmer et al., 2010).*
- The leaves, flowers, and berries of hawthorn contain a variety of flavonoids which include oligomeric procyanidins, vitexin, quercetin, and hyperoside *(Qiao et al., 2015).*
- Hawthorn has a long history of use in traditional Chinese medicine (TCM) and European herbal medicine *(Chang et al., 2002).*
- Nowadays hawthorn extracts are among the most popular herbal medicinal products in many European countries and the USA after getting attention due to their potential cardiovascular enhancing and protective properties.
- Ultrasound is one of the environmentally friendly extraction methods, Compared to traditional methods, UAE has advantages such as less time and energy requirement, extraction at low temperature, and retention of the quality of the extract *(Kumara et al., 2021).*

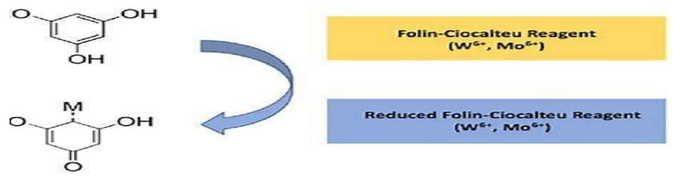


- At present, the economical feasibility of an industrial process also requires working in such a way that high extraction efficiency is attained. Many factors have been established to improve the extraction efficacy, such as temperature, time, and solvent type. When several factors and their potential interactions may affect the desired response, response surface methodology (RSM) is an effective tool to optimize the process. RSM has been very popular for optimization studies in recent years. In fact, RSM has been used to optimization of the extraction process of plant materials *(Aydar et al., 2018).*

MATERIALS AND METHODS:

- **Hawthorn fruit was collected from Normafa, Hungary**.
- **Total phenolic compounds (Folin-Ciocalteu method):**

The total phenolic compounds (TPC) was performed by the Folin-Ciocalteu method *(Zin et al.,2020).*

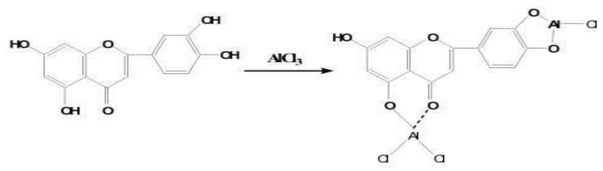


- **Antioxidant activity (AA) assays (FRAP Method):**

The ferric reduction antioxidant power (FRAP) method was applied to quantify AA in the bio-extracts by a simple redox reaction in which the reduction of ferric ions to ferrous ions with intensive color changes serves as an indicator *(Benzie et al.,2018).*

- **Total flavonoid compounds (Aluminium chloride assay):**

The measurement of the total flavonoid content (TFC) of the extracts was accomplished by aluminium chloride assay based on the method of Ardekania *(Ardekania et al., 2011).*



STATISTICAL ANALYSIS:

Statistical analysis was implemented with DOE by the design of expert software (version 11.0.3). The Central-Composite design under the response surface methodology (RSM) was used to optimize the process of extraction from hawthorn. Extraction time (A), and sample ratio (B) were the two independent variables, which were studied at three different levels (-1, 0, 1). The actual factors levels are shown in Table 1.

Table 1. The actual factors levels table

Variable	Factor	Level		
		-1	0	1
(A)	Extraction time (min)	3	6	9
(B)	Sample ratio %	5	7.5	10

RESULTS:

- The results showed that two independent variables, time of extraction (A), and the sample ratio(B), both had significant effects ($p<0.05$). the time of extraction (A) was the most significant ($p < 0.0001$) factor affecting TPC by using distilled water and ethanol 10%, while the sample ratio (B) was the most significant ($p < 0.0001$) factor affecting AA. The value of the determination coefficient R-Squared for TFC and AA in both cases (distilled water and ethanol 10%) indicate the fitting degree of the equation is promising. the following regression equations are built by the model with coded units to predict the values of dependent variables:

For distilled water extracts:

- $Sqrt(TFC) = 1.057 + 0.204 \cdot A + 0.019 \cdot B - 0.0125 \cdot A \cdot B - 0.008 \cdot A^2$
- $Sqrt(TPC) = 1.761 + 0.051 \cdot A + 0.036 \cdot B$
- $Sqrt(AA) = 1.571 + 0.035 \cdot A + 0.099 \cdot B - 0.01 \cdot A \cdot B + 0.004 \cdot A^2$

For ethanol 10% extracts:

- $Sqrt(TFC) = 1.714 + 0.163 \cdot A + 0.033 \cdot B - 0.013 \cdot A^2$
- $Sqrt(TPC) = 1.835 + 0.202 \cdot A + 0.026 \cdot B - 0.013 \cdot A^2$
- $Sqrt(AA) = 2.18 + 0.069 \cdot A + 0.031 \cdot B - 0.005 \cdot A^2$

Whereas; A means extraction time and B means sample ratio.

- The effects of variables and their interactions on the extraction rate of TPC, TFC, and AA were elucidated by the three-dimensional (3D) response surface observations and two-dimensional (2D) contour plots (Figure 1 (a,b)). The interaction effects between the variables were exhibited by the shapes of the contour plots.
- Table (2) shows the best conditions obtained for the extraction process using distilled water and ethanol 10% and TPC, TFC, and AA under these conditions.

Table 2: The best conditions for extraction process

Solvent	Time (min)	Solvent ratio (%)	TFC (mg QUE/g DM)	TPC (mg GAE/g DM)	AA (mg ASE/g DM)	Desirability
DW	7.423	10.000	5.482	6.26	5.577	0.804
Eth-water	7.219	9.922	6.497	8.397	7.484	1

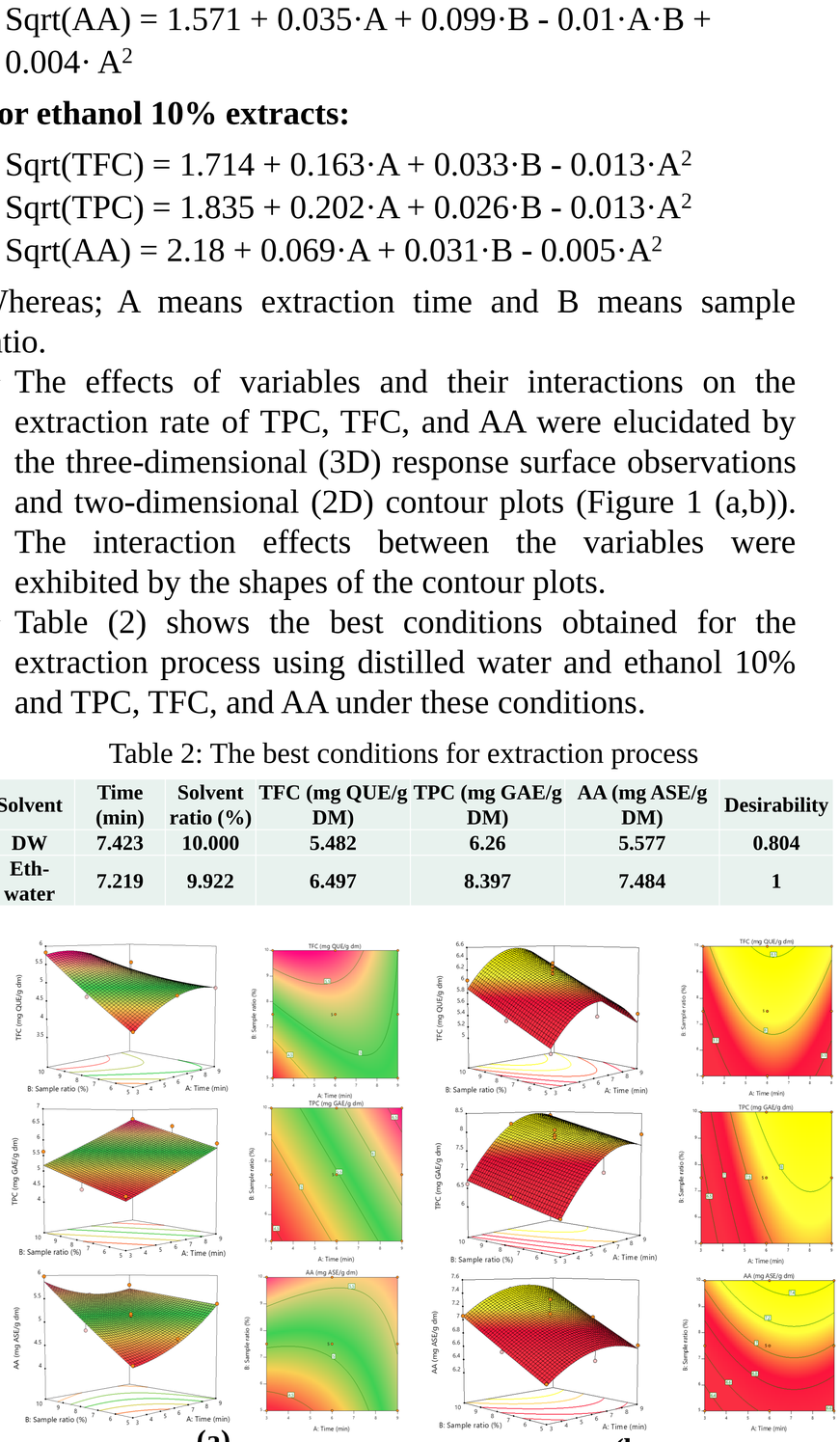


Figure (1) Response surface and contour plots of the influence of extraction time and solvent ratio on TPC, TFC, and AA using distilled water (a) and ethanol 10% (b)

CONCLUSIONS:

The Central-Composite experiment design optimized the extraction process for hawthorn, the optimal extraction condition was found to be at: 7.423 minutes, 10 g of the sample/100 mL distilled water as a solvent, and 7.219 minutes and 9.922 g of the sample/100 mL 10 v/v% ethanol as solvent. Under these conditions, the obtained extracts exhibited content of phenolic compounds (6.26 and 8.397 mg gallic acids equivalents/g dry matter), the content of flavonoids compound (5.482 and 6.497 mg quercetin equivalents/g dry matter), with antioxidant activity (5.577 and 7.484 mg ascorbic acid equivalents/g dry matter) by using distilled water and 10 v/v% of ethanol, respectively.

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