Berberis crataegina DC. as a novel natural food colorant source: ultrasound-assisted extraction optimization using response surface methodology and thermal stability studies

Mehmet DEMIRCI1,2*, Merve TOMAS1, Zeynep Hazal TEKIN-ÇAKMAK1, Salih KARASU2* 1

Abstract
This study aimed to investigate the potential use of anthocyanin of Berberis crataegina DC. as a natural food coloring agent in the food industry. For this aim, the ultrasound-assisted extraction (UAE) method was performed to extract anthocyanin of Berberis crataegina DC. The effect of ultrasound power (X1: 20-100%), extraction temperature (X2: 20-60 °C), and time (X3: 10-20 min) on TPC and TAC of Berberis crataegina DC. extracts were examined and optimized by applying the Box–Behnken experimental design (BBD) with the response surface methodology (RSM). The influence of three independent variables and their combinatorial interactions on TPC and TAC were investigated by the quadratic models (R2: 0.9638 & 0.9892 and adj R2: 0.9171 & 0.9654, respectively). The optimum conditions were determined as the amplitude level of 98%, the temperature of 57.41 °C, and extraction time of 13.86 min. The main anthocyanin compounds were identified, namely, Delphinidin-3-O-galactoside, Cyanidin-3-O-glucoside, Cyanidin-3-O-rutinoside, Petunidin-3-O-glucoside, Pelargonidin-3-O-glucoside, and Peonidin-3-O-glucoside. The anthocyanin degradation showed first-order kinetic, degradation rate constant (k), the half-life values (t1/2), and loss (%) were significantly affected by different temperatures (P < 0.05). Higher degradation (k) in anthocyanin content was observed at 90 °C. This study suggested that UAE is an efficient method for the extraction of TPC and TAC from Berberis crataegina DC.

Keywords: Berberis crataegina DC., ultrasound-assisted extraction, box-behnken experimental design, anthocyanin, natural food colorant.

Practical Application: The ultrasound-assisted extraction optimization of Berberis crataegina DC.

1 Introduction
Berberis crataegina DC. are fruits of wild plant belongs to the Berberidaceae family, grown commonly in the Anatolia region of Turkey. The Berberis crataegina DC. are consumed widely in raw and or after being processed into natural juices, marmalades, and jellies, and dried in the sun (Akbulut et al., 2009; Işıklı & Yılmaz, 2014; Mutalleb et al., 2005). The color of Berberis crataegina DC. varies from dark purple to black and their tastes are slightly sour due to the berberine content of fruits (Baytop, 1963; Eroğlu et al., 2020; Siow et al., 2011). The Berberis crataegina DC. has organic acids, high content of vitamin C, tannins, and high anthocyanin content. Therefore, they have a strong antifungal activity and anti-inflammatory, anti-irritating, and diuretic effects antioxidant and free radical scavenging properties due to phenolic compounds especially anthocyanin (Ullah et al., 2015; Yeşilada & Küpeli, 2002). Several investigations have been conducted to evaluate the phenolic compounds, antioxidant activities, and organic acid contents of Berberis crataegina DC. (Charehsaz et al., 2015; Gulsoy et al., 2011), which is rich in polyphenols (Eroğlu et al., 2020) and anthocyanin content. However, no study has been conducted on potential use of natural colorant of anthocyanin extracted from Berberis crataegina DC. The anthocyanin naturel colorant (Brouillard et al., 1982). The anthocyanin stability is mostly influenced by temperature, pH, chemical structure, and light (Castañeda-Ovando et al., 2009). Therefore, the extraction of anthocyanins is a crucial step for the isolation, identification, and use of anthocyanin as a food colorant.

Extraction techniques applied for bioactive compounds recovery from fruits and plants may differ considering the extraction yield and production cost. Traditional extraction methods require a significant amount of energy and solvent, and an intensive labor force. Also, these methods can degrade thermally sensitive compounds such as anthocyanin (Sharifi & Hassani, 2012). The UAE method shortens the extraction time and energy requirements as compared to conventional extraction methods. Also, the UAE method provides the recovery of bioactive compounds through the action of acoustic cavitation generated by an ultrasound wave in the solvent, and the enhancement of mass transport by disrupting the plant cell walls (Dranca & Oroian, 2016; Li et al., 2019; Riciputi et al., 2018; Rodrigues et al., 2015). On the other hand, the UAE efficiency is affected by several parameters such as ultrasound amplitude, extraction time, temperature, solvent-sample ratio, and pH (Chemat et al., 2017). Therefore, the optimization of the extraction process is necessary to obtain high-yield and high-quality bioactive compounds. For the optimization, the effects of the selected parameters on the extraction efficiency analyze through a Box–Behnken experimental design (BBD).
with a response surface methodology (RSM), which consists of mathematical techniques to maximize the analytical response (Ding et al., 2016; Li et al., 2019). However, to the best of our knowledge, there is no literature on the UAE of TPC and TAC from Berberis crataegina DC. Also, it is necessary to optimize the conditions of the UAE of Berberis crataegina DC. to determine the potential use as a natural food coloring agent because there is no study about the potential sources of natural food coloring agents of Berberis crataegina DC. for the food industry. However, the chemical structure of anthocyanin pigments is not stable and they are susceptible to degradation during thermal processing (Kara & Erçelebi, 2013). Therefore, it is essential to determine the kinetic parameters for prediction of the quality changes.

The food industry uses food coloring agents to determine the acceptability of processed food to consumers. Okafor et al. (2016) studied about the development of the global food coloring market. They stated that the demand for food color agents in the global market was 2400 Metric Tons, and it was expected to increase to 15,000 Tons in 2015. However, there is a growing demand for natural food color agents as there is a growing concern about the health effects of the use of synthetic food color agents in human diets. Therefore, the UAE of TPC and TAC from Berberis crataegina DC. was studied for the determination of the potential of new source of the natural food colorant. The objective of the study is to investigate the optimum extraction conditions of independent variables, namely the ultrasound amplitude, extraction temperature, and time using RSM and to determine the thermal stability of the anthocyanin.

2 Material and method

In this study, Berberis crataegina DC. grown under natural conditions without cultivation in Tunceli province was collected in August. The collected fruits were transferred to the laboratory then cleaned and stored in a refrigerator at 4 °C until analysis. The chemicals used in the study were obtained from Merck (Merck, Darmstadt, Germany).

2.1 Experimental Design of Extraction

A three-level Box–Behnken experimental design (BBD) with a response surface methodology (RSM) was performed to optimize the ultrasound-assisted extraction (UAE) conditions of Berberis crataegina DC. (Fernandez-Barbero et al., 2019). In this experimental design, three independent variables at three different levels, i.e., (-1) low, (0) medium, and (+1) high, were studied. The independent variables and their levels of the BBD were given in Table 1. In determining these specific ranges of values, we have benefited from the existing knowledge on the extraction of phenolic compounds and anthocyanins previously studied (Karasu et al., 2019; Skrypnik & Novikova, 2020). The response variables were TPC and TAC of the Berberis crataegina DC. Seventeen different experimental points with three center points for each of the three replicates were performed.

2.2 UAE Procedure of Berberis crataegina DC

In the UAE procedure, Berberis crataegina DC. were extracted by using an ultrasonic bath (WiseClean, DH.WUC.D10H, Germany—40 kHz, 258 W) continuously. Berberis crataegina DC. (5 mg) were extracted with 50 ml methanol-water (80:20) according to the conditions specified in the experimental design (Table 1) and homogenized by Ultra-Turrax (Daihan, HG-15D, South Korea) at 10,000 rpm for 3 min. Then, the extracted samples were centrifuged at 4500 rpm and the centrifuged samples were first roughly filtered and then filtered through 0.25 μm filters. The obtained extracts were stored at 4 °C until further analysis (Gazeran et al., 2016).

### Table 1. Independent variables and levels of variables for the Box-Behnken design.

<table>
<thead>
<tr>
<th>Symbols</th>
<th>Independent Variables</th>
<th>Levels</th>
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<tr>
<td>X₁</td>
<td>Power, A</td>
<td>-1</td>
</tr>
<tr>
<td>X₂</td>
<td>Temperature, °C</td>
<td>20</td>
</tr>
<tr>
<td>X₃</td>
<td>Time, min</td>
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<thead>
<tr>
<th></th>
<th>0</th>
<th>60</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

2.3 Determination of TPC and TAC of Berberis crataegina DC

TPC in Berberis crataegina DC. extracts was determined with Folin–Ciocalteu method (Singleton & Rossi, 1965). The absorbance values were measured at 765 nm (UV-3600, Shimadzu, Kyoto, Japan). TPC was expressed as mg gallic acid equivalent per 100-gram.

TAC in Berberis crataegina DC. extracts was determined by using the pH differential method (Fuleki & Francis, 1968). Absorbance values were measured at 517 nm with a spectrophotometer (UV-3600, Shimadzu, Kyoto, Japan). TAC was expressed as mg per L.

2.4 Determination of the phenolic acid and anthocyanin profile of Berberis crataegina DC

Individual phenolic acid and anthocyanin profile of ultrasound-assisted Berberis crataegina DC. extracts were identified by HPLC coupled to a diode array (HPLC-DAD, Shimadzu Corp., Kyoto, Japan). The extracts were obtained for TPC and TAC analysis and they were filtered through a 0.45-μm membrane filter. Then, 1 ml of the filtered sample was analyzed in an HPLC system (LC-20AD pump, SPDM20A DAD detector, SIL-20A HT autosampler, CTO-10ASVP column oven, DGU-20A5R degasser, and CMB-20A communications bus module (Shimadzu Corp., Kyoto, Japan). Separations were conducted at 40 °C on a reversed-phase column (Intersil® ODS C-18, GL Sciences, Tokyo, Japan) with a 250mm×4.6mm length, 5 μm particle size. The mobile phases were solvent A (distilled water with 0.1% (v/v) acetic acid) and solvent B (acetonitrile with 0.1% (v/v) acetic acid). The flow rate was set as 1 ml/min and the chromatograms were recorded at 254–356 nm. Twelve phenolic acids as standards, 4-Hidroksibenzoik acid, Coumaric acid, Cinnamic acid, Syringic acid, Vanillic acid, Chlorogenic acid, Ferulic acid, Sinapinic acid, Gentisic acid, Trans-3-hydroxy cinnamic acid, Caffeic acid, and Gallic acid, were used for phenolic acids separation and quantification. Also, six anthocyanins as standards, Delphinidin-3-O-galactoside, Cyanidin-3-O-
glucoside, Cyanidin-3-O-rutinoside, Petunidin-3-O-glucoside, Pelargonidin-3-O-glucoside, Peonidin-3-O-glucoside, were used for anthocyanins separation and quantification. The result of individual phenolic acid and anthocyanin amounts were expressed as mg/100 g.

2.5 Thermal degradation kinetics of anthocyanin

The thermal stability of anthocyanin from Berberis crataegina DC. was studied at 70, 80, and 90 °C (Wang & Xu, 2007). The anthocyanin content was determined for every 1 h interval. The thermal degradation data were fitted to the exponential kinetic model to determine the first-order kinetics behavior of the anthocyanin of Berberis crataegina DC. by Equation 1:

\[ C = C_0 \times \exp(-k \times t) \]  

where \( C_0 \) is the initial anthocyanin content, \( C \) is the anthocyanin concentration of Berberis crataegina DC. after heating at a given temperature, and \( k \) is the constant for the first-order kinetic model. Half-time \((t_{1/2})\) was calculated using Equation 2 as the following:

\[ t_{1/2} = \frac{\ln(0.5)}{k} \]  

2.6 Statistical analysis

All experiments were carried out in triplicates. The experimental results were expressed as means ± standard deviation. A one-way ANOVA test was used in the SPSS program. Duncan’s multiple test range was applied for comparisons of means. A probability level of \( p < 0.05 \) was considered statistically significant.

3 Results and discussion

3.1 UAE of Berberis crataegina DC

TPC and TAC extraction from Berberis crataegina DC. was performed using ultrasonic treatment (20, 60, and 100 A, respectively) by varying temperature (20, 40, and 60 °C) and extraction time (10, 15, and 20 min) for the optimization of extraction yield. The experiment was conducted in seventeen runs to investigate the effect of the selected variables on TPC and TAC values obtained under different experimental conditions were presented in Table 2.

Our results showed that TPC values ranged from 10.76 to 62.95 mg/100 g and TAC values ranged from 785.234 to 2659.297 mg/L. The highest TPC (more than 34 mg/100 g) and TAC (more than 2000 mg/L) values were obtained at 100 A ultrasound power, and 40 and 60 °C. Ghafoor et al. (2009) studied the experimental values of total phenols, antioxidant activities, and anthocyanins of grape seed extract obtained by UAE at the experimental conditions (ethanol concentration, extraction time, and temperature) in eighteen runs. Also, Bing et al. (2019) studied that the ultrasound-assisted extraction (UAE) of sheep abomasum protein concentrates (SAPC) and the investigation of the properties of SAPC. They reported that the highest yield and protein values in SAPC obtained by the UAE were 24.41 ± 0.71% and 92.36 ± 3.80 g/100 g, respectively.

The quadratic model modeled the influence of the employed parameters on TAC and TPC extraction, as well as the interactions between parameters were presented in Table 3.

Estimated coefficients of the linear, quadratic, and interaction factors of the polynomial equations and their significance (p-values) were evaluated. According to Table 3, the linear and quadratic terms were highly significant (\( P < 0.01 \)), indicating that the model could adequately fit the experiment data. All individual parameters significantly affected TPC and TAC (\( P < 0.1 \)). According to Table 3, R² and adj R² values were 0.9892 and 0.9654 for TPC and 0.9638 and 0.9171 for TAC, and the lack of fit was insignificant (\( P > 0.05 \)). This result indicated that the quadratic model successfully described the effect of extraction process parameters on TPC and TAC. In contrast, the interaction between the ratio of ultrasound power and extraction temperature \((X_1 X_2)\), the quadratic term of extraction temperature \((X_2^2)\), and the quadratic term of extraction time \((X_3^2)\) were not significant. According to Table 3, the model parameters of ultrasound power showed a smaller P-value and significant effect on the TPC and TAC. Therefore, optimization of ultrasound power is an important step. Chen et al. (2007) reported that the change of ultrasonic power and extraction time had a significant effect on the TAC of red raspberries extracts. In our study, at a low level of extraction time (13.86) and temperature (57.41 °C), maximum desirability was obtained.

Three-dimensional response surface plots, which showed the effect of process parameters and the interaction of these parameters on TPC, were presented in Figure 1. There is a linear increase in TPC with an increase in extraction time at a fixed amplitude and temperature (Figure 1b, c).

Table 2. Experimental values of TPC and TAC of Berberis crataegina DC. obtained from Box-Behnken design.

<table>
<thead>
<tr>
<th>Run</th>
<th>( X_1 )</th>
<th>( X_2 )</th>
<th>( X_3 )</th>
<th>TPC (mg/100g)</th>
<th>TAC (mg/L)</th>
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<tr>
<td>1</td>
<td>20</td>
<td>20</td>
<td>10</td>
<td>10.76</td>
<td>785.234</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>20</td>
<td>15</td>
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<td>3</td>
<td>100</td>
<td>60</td>
<td>10</td>
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<td>20</td>
<td>40</td>
<td>15</td>
<td>14.33</td>
<td>1524.480</td>
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<tr>
<td>6</td>
<td>100</td>
<td>40</td>
<td>15</td>
<td>46.31</td>
<td>2247.670</td>
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<tr>
<td>7</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>11.44</td>
<td>1004.440</td>
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<tr>
<td>8</td>
<td>60</td>
<td>40</td>
<td>15</td>
<td>23.20</td>
<td>1967.380</td>
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<tr>
<td>9</td>
<td>100</td>
<td>40</td>
<td>10</td>
<td>34.00</td>
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<tr>
<td>10</td>
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<td>15</td>
<td>24.83</td>
<td>1658.199</td>
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<td>11</td>
<td>100</td>
<td>40</td>
<td>20</td>
<td>62.95</td>
<td>2463.980</td>
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<td>12</td>
<td>60</td>
<td>60</td>
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<td>15</td>
<td>22.21</td>
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<td>14</td>
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<td>16</td>
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<td>17</td>
<td>60</td>
<td>40</td>
<td>20</td>
<td>30.82</td>
<td>1670.140</td>
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*Run number was used for identification purposes only and does not indicate the order in which the experimental runs were conducted.

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Ramić et al. (2015) similarly reported that the ultrasound-assisted extracts of polyphenolic compounds from Aronia melanocarpa by-products from filter-tea factory had a higher yield of TPC as the extraction time increased. It is noticeable that the ultrasound power dominantly affects TPC comparing to extraction time and temperature, by analyzing the slopes for each variable.

Figure 2 showed the response surface plots of multiple influences of all independent variables that confirmed the mathematical analysis of UAE of TAC. Figure 2b, c showed a linear increase in TAC with an increase in extraction time at a fixed ultrasound power and temperature. Similarly, there is a linear increase in TAC with an increase in extraction temperature from 20 °C to 60 °C at a fixed ultrasound power and time and with an increase in ultrasound power at a fixed extraction time and temperature. This positive quadratic effect of temperature on TAC can be explained that the increase of temperature can accelerate the molecular mass transfer, the conductivity of

<table>
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<th>Regression coefficients</th>
<th>df</th>
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<th>F</th>
<th>p</th>
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<td>X₂ X₃</td>
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<td>0.0582</td>
<td>594.71</td>
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<td>X₁²</td>
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*df* degree of freedom values of “Prob > F” less than 0.05 indicate model terms are significant.

Figure 1. 3D surface and counterplot for TPC.
the solution to the plant material. Therefore, the influence of temperature can increase the solubility and diffusivities of the plant materials into the solution so that the extraction yield can increase (Yang et al., 2010). Also, the temperature has a greater effect on the cavitation threshold, which causes acoustic cavitation and results in cavitation core formation. The effect of the relatively greater shear force shattered and exploded the cavitation nucleus formed and broke cell walls during extraction, which increased the mass transfer rate (Toma et al., 2001) and increased extraction efficiency (Figure 2a,b).

As can be seen in Figure 2, the ultrasound power dominantly influences TAC comparing to extraction temperature and time, by analyzing the slopes for each variable (Figure 2a, c). Therefore, it is expected that the upper level of power (100A) will be optimal for the extraction of TAC. Ghafoor et al. (2009) observed that a linear increase in total anthocyanin contents with an increase in extraction temperature on grape seed anthocyanins at a fixed ethanol concentration (50%).

### 3.2 Optimization of extraction parameters

The statistical optimization of UAE of TPC and TAC was performed by using the RSM-generated model. Celli et al. (2015) was preferred this model for the optimization of TAC of haskap berries. In this study, ultrasonic treatment (20, 60, and 100 A, respectively), extraction temperature (20, 40, and 60 °C), and extraction time (10, 15, and 20 min) were selected as factors. The optimum extraction conditions were determined based on the maximum response of TPC and TAC of *Berberis crataegina* DC. extracts obtained by UAE according to the quadratic regression equation obtained from the Design Expert 7.0 software program. The optimum extraction parameters were determined as 57.41 °C, 13.86 min, and 98.00 amplitude levels. TPC and TAC were also determined experimentally for verification of statistical results at the experimental point. Arici et al. (2016) determined that the optimum extraction temperature and time combination for tulip anthocyanin were 54 °C and 116 min.

### 3.3 Phenolic acid and anthocyanin profile of the optimized extract

Table 4 showed the concentration of phenolic compounds obtained from UAE of *Berberis crataegina* DC. extract prepared at the optimal conditions. Six anthocyanin compounds were identified, namely Delphinidin-3-O-galactoside (82.05 mg/100g), Cyanidin-3-O-glucoside (85.48 mg/100 g), Cyanidin-3-O-rutinoside (49.35 mg/100 g), Petunidin-3-O-glucoside (1.48 mg/100 g), Pelargonidin-3-O-glucoside (21.93 mg/100 g) and Peonidin-3-O-glucoside (50.91 mg/100g) were determined. Although different fruits and vegetables contain different anthocyanin compounds, the predominant anthocyanins include in fruits and vegetables are cyanidin-3-O-glucoside, delphinidin-3-O-glucoside, malvidin-3-O-glucoside, pelargonidin-3-O-glucoside, petunidin-3-O-glucoside have been used to obtain color varied from red to purple and blue color (Martins et al., 2016). Therefore, *Berberis crataegina* DC. may be evaluated as a new source of food colorant in the food industry. Celli et al. (2015) determined that five anthocyanins of the optimized extract obtained by UAE in haskap berries were identified, namely cyanidin 3,5-diglucoside, cyanidin 3-glucoside,
cyanidin 3-rutinoside, petunidin 3-glucoside, and peonidin 3-glucoside. As shown in Table 4, Cyanidin-3-O-glucoside (85.48 mg/100 g) was determined as the major anthocyanin of Berberis crataegina DC. While Petunidin-3-O-glucoside (1.48 mg/100 g) was determined as a minor anthocyanin of Berberis crataegina DC. A similar result was reported for red onions by Patras et al. (2010). Major anthocyanin was identified as cyanidin 3-glucoside for red onions while minor anthocyanins were delphinidin 3-glucoside and petunidin glucoside.

Twelve phenolic acids were identified, namely 4-Hidroksibenzoik acid, Coumaric acid, Cinnamic acid, Syringic acid, Vanillic acid, Chlorogenic acid, Ferulic acid, Sinapinic acid, Gentisic acid, Trans-3-hydroxy cinnamic acid, Caffeic acid, and Gallic acid. Also, the extract of Syrah grape skin were catechin, epicatechin, quercetin, gallic acid, and p-coumaric acid. The magnitude and duration of heating, the combination of unit operations involving heat, and the inclusion of a blanching step for the prevention of enzyme degradation affect strongly the stability of anthocyanin (Patras et al., 2010). Previous studies showed the thermal degradation of blackberry anthocyanins were decreased from 170.045 min to 80.018 min with increasing temperature from 70 °C to 90 °C.

As shown in Table 5, the half-life time (t1/2) values ranged from 170.045 min to 80.018 min with increasing temperature. The k values were 0.407*10⁻¹ min⁻¹, 0.522*10⁻¹ min⁻¹, and 0.866*10⁻² min⁻¹ at 70, 80 and 90 °C, respectively. The increase of k value with increasing temperature was explained that anthocyanin degradation was greatly dependent on temperature (Kirca et al., 2003). Yang et al. (2008) reported that the coefficients of determination (R²) values were between 0.937 and 0.974 and the degradation rate of aqueous anthocyanins of purple corn cob increased with increased temperature from 70 °C to 90 °C.

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according to findings of the extraction and thermal stability. Ultrasonic waves can efficiently improve the extraction of bioactive compounds. UAE parameters significantly affected TPC and TAC. The RSM was successfully employed to optimize the extraction and the experimental parameters (ultrasound power, extraction temperature, and time) have been evaluated. This study suggested that anthocyanin content could be increased at 100% ultrasound power, and 40 and 60 °C. Also, the degradation of anthocyanin during the thermal process followed first-order reaction kinetics and the degradation rate increased as temperature increased. The percentage loss of Berberis crataegina DC. anthocyanin was determined as 33.607%, 36.399%, and 56.765% at 70, 80, and 90 °C, respectively. When considering their anthocyanin content during mild heat treatment (40 and 60 °C), Berberis crataegina DC. may be developed as a potential natural colorant for the pharmaceutical and food industries.

References


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Assessment of the UAE of anthocyanin of Berberis crataegina DC


