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Ultrasound assisted anthocyanin extraction from sumac and investigation of thermal degradation kinetics of the anthocyanins

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Abstract

In this study, an ultrasonic extraction method was used to extract anthocyanins from sumac. In order to maximize the yield of anthocyanins from the extraction, a three-factor Taguchi method with ethanol concentration (25, 50, 75%), sonication time (15, 30, 45 min), and solvent: solute ratio (10:1, 20:1, 30:1) was used. Under optimum extraction conditions, maximum anthocyanin yield (632.024 mg C3G/kg) was obtained by performing 15 min sonication of the mixture prepared with 75% ethanol at 10:1 solvent: solute ratio. The solvent: solute ratio was the parameter that most affected the extraction process. However, as a result of ANOVA analysis, the effect of the ethanol concentration variable on the extraction process was found to be significant ($p < 0.05$). In this study, the thermal degradation kinetics of sumac anthocyanins obtained under optimum extraction conditions as a result of 70, 80, and 90 °C heat treatment were also determined. The results showed that the thermal degradation process fit the first-order reaction kinetics. The kinetic rate constant (k) of thermal degradation of sumac anthocyanins varied between $1.69x10^{-3}$-$5.44x10^{-3}$ min^{-1} and the half-life ($t_{1/2}$) varied between 29.46-9.15 hrs. The activation energy (Ea) of thermal degradation of sumac anthocyanins was calculated as 60.28 kJ/mol. Taguchi method was successfully applied in the extraction of anthocyanins from sumac. There is an urgency to improve the stability of the extracted sumac anthocyanins by considering the food production conditions.

Key Words: *Sonication, kinetic rate constant, half-life, activation energy*

Introduction

Sumac (*Rhus coriaria*) naturally grows in the wild stretching from the Canary Islands across the Mediterranean coastline to regions in Iran and Afghanistan. The plant is indigenous to the Mediterranean area and southeastern Anatolia in Turkey. In the Middle East and Mediterranean regions, Sumac has been employed for a variety of traditional purposes, such as spice, dye, and medicinal herbs. Rhus coriaria is a valuable source of nutritional components, including organic acids, both essential and non-essential fatty acids, vitamins, carbohydrates, and minerals (K, Ca, Mg, Na, P, Fe). Additionally, it contains tannins, phenolic acids, anthocyanins, flavonoids, and terpenoids (Ozcan et al., 2021). Notably, extracts derived from sumac have exhibited antimicrobial, antifungal, and antiviral properties (Romeo et al., 2015).

Anthocyanins are widely utilized as natural colorants in the food industry. These natural pigments can exhibit a broad range of colors, ranging from orange to red, and purple to blue, depending on their molecular structure and pH levels. The interest in anthocyanins stems not only from their coloring effects but also from their health benefits. Due to increasing environmental concerns associated with synthetic dyes, natural dyes offer an environmentally friendly alternative for coloring in the food and pharmaceutical sectors (Ravanfar et al., 2015).

Nowadays, consumers are demanding the use of these natural colorants with beneficial health effects in food products. Anthocyanins, which are recognized as natural food colorants in the fruit and vegetable category in the US, are classified as e163 in the EU. Although there are many identified anthocyanin compounds, only pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin are prevalent in fruits and cereal grains. These are active compounds with antioxidant activity and health-promoting properties owing to their anti-inflammatory and hypoglycemic effects. They also exhibit antimutagenic and anticarcinogenic activity (Loypimai et al., 2016).

It is well known that factors such as solvent type, liquid-solid ratio, and extraction time significantly influence the yield and bioactivities of active substances extracted from plants. However, the influence of these extraction parameters can vary depending on several factors, especially the specific plant material used in the extraction process. The conventional approach of one-factor-at-a-time experiments is both time-consuming and does not provide insight into the interactions between factors. Alternatively, Taguchi's methodology is highly effective in screening procedures to identify important factors whilst minimizing the number of experiments required (Ashengroph et al., 2013). A review of the existing literature shows that Taguchi modeling has not been applied to the extraction of anthocyanins from sumac. Additionally, there is a lack of data concerning the thermal degradation kinetics of anthocyanins obtained from sumac. Thus, this study has two main objectives: first to

determine the optimal conditions for extracting anthocyanins from sumac spice using ultrasonic extraction, focusing on three key process factors including ethanol concentration, sonication time, and solvent: solute ratio, through the Taguchi method. Second, to investigate the degradation kinetics of the extract with the highest anthocyanin content under optimal conditions when subjected to heat treatments at temperatures of 70, 80, and 90 °C. This investigation includes the determination of reaction rate constant (k), half-life ($t_{1/2}$), and activation energy (Ea).

Materials and Methods

Material

Sumac used in this study was obtained in spice form from a local supplier in Pamukova district of Sakarya. The chemicals used in the analysis were of analytical purity and were obtained from Merck (Germany).

Extraction procedure and Taguchi optimization

The sumac oil was extracted using petroleum ether in a soxhelet extraction unit for 8 hours (Kosar et al., 2007). Then, the required amount of the dried sample (solvent: solute ratio (10:1, 20:1, 30:1)) was taken and mixed with 100 ml of solvent containing different concentrations (25, 50, 75%) of ethanol. The sample mixture was extracted in an ultrasonic water bath at a frequency of 40 kHz for different times (15, 30, and 45 min). The temperature was kept below 30 °C during the extraction process. Ice sockets were used for this purpose. The sample mixtures were then firstly removed from the coarse elements using a fourfold cheesecloth and then the filtrate was filtered through a water trombe using Whatman no:1 filter paper. The clear extracts were kept at refrigerator temperature until analysis.

In this study, Taguchi L9 orthogonal experimental design was used to maximize anthocyanin extraction by minimizing the number of experiments. The factors and levels utilized in anthocyanin extraction from sumac are presented in Table 1. The effect of ethanol concentration (A), sonication time (B), and solvent: solute ratio (C) on maximum anthocyanin extraction was investigated. The anthocyanin extraction process was optimized using the Taguchi L9 experimental design in Table 2.

In the Taguchi optimization method, the effect of each extraction parameter on anthocyanin extraction is tested by signal-to-noise ratio (S/N). In general, S/N ratios are evaluated in 3 categories "nominal best", "smallest best" and "largest best" (Güldane, 2023). In this study, the S/N ratios analysis was performed by selecting the "largest best" (Equation 1) since it was aimed to maximize the anthocyanin content.

$$S/N = -10log\left[1/R \sum_{i=1}^{R} 1/y_i^2\right] \quad (1)$$

where R is the number of data points and y_i is the value of the i^{th} data point.

Table 1. Factors and corresponding levels for design matrix

Factors	Unit	Symbol	Level 1	Level 2	Level 3
Ethanol concentration	%	A	25	50	75
Sonication time	min	B	15	30	45
Solvent: solute ratio	-	C	10:1	20:1	30:1

Table 2. Taguchi L9 design matrix for extraction of sumac anthocyanins, experimental results, and S/N ratios

Run	Ethanol concentration (%)	Sonication time (dak)	Solvent: solute ratio	Total anthocyanin (mg C3G/kg)	S/N (dB)
1	25	15	10:1	514.682	54.2308
2	25	30	20:1	367.953	51.3158
3	25	45	30:1	252.566	48.0475
4	50	15	20:1	434.672	52.7632
5	50	30	10:1	240.589	47.6255
6	50	45	30:1	577.631	55.2330
7	75	15	10:1	339.069	50.6058
8	75	30	30:1	619.379	55.8391
9	75	45	20:1	488.026	53.7689

Determination of total monomeric anthocyanin (TMA) content

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TMA content of sumac extracts was determined by pH differential method using a UV-VIS spectrophotometer (Shimadzu UV-1240, Japan). The clear anthocyanin extract was diluted with a pH 1.0 buffer solution (0.025M KCl) and pH 4.5 sodium acetate buffer solution (0.4M CH_3COONa-$3H_2O$) in separate test tubes. The absorbance of each sample was read at 510 and 700 nm against distilled water. The absorbance (Abs) value of the diluted sample was determined using Equation 2.

$$Abs = (A_{510\ nm} - A_{700\ nm})_{pH\ 1.0} - (A_{510\ nm} - A_{700\ nm})_{pH\ 4.5} \quad (2)$$

The TMA content of the samples was determined according to the following formula (Equation 3).

$$TMA\ \left(\frac{mg}{kg}\right) = \frac{Abs * M_w * DF * 1000}{\varepsilon * l} \quad (3)$$

where M_w is the molecular weight for cyanidin-3-glucoside (C3G) (445.2), DF is the dilution factor value, and ε is the molar absorbtivity constant for C3G (26,900) (Kirca et al., 2006).

Thermal degradation kinetics of sumac anthocyanins

Thermal degradation of sumac anthocyanins at 70, 80, and 90 °C was investigated by a slightly modified method proposed by Kirca and Cemeroğlu (2003). 10 ml of the extract liquid containing 632 ± 5.48 mg C3G/kg sample level of anthocyanins was transferred into 10 ml test tubes. After the test tubes were sealed, they were placed in heat-resistant tube spores and placed in a thermostatic water bath. At 30 min intervals, a random tube was taken and rapidly cooled in the ice water bath. Sample analysis times were 360, 360, and 270 min for 70, 80 and 90 °C. The cooled samples were kept at 4 °C until analysis.

Previous studies have shown that the degradation process of color pigments from various natural sources fits the first-order reaction model. In this study, the reaction degree fitting the degradation process of sumac color pigments was determined by regression analysis. In this study, the reaction rate constant (k, min^{-1}) (Equation 4) was used to calculate the time required for the degradation of half of the anthocyanins ($t_{1/2}$) (Equation 5). Furthermore, the temperature dependence of the degradation rate constant was determined by applying the Arrhenius equation (Equation 6). The activation energy (Ea) was calculated by plotting ln (kT) versus 1/T (Chen et al., 2019).

$$C_t = C_0 \exp(k.t) \quad (4)$$

$$t_{1/2} = -ln0.5/k \quad (5)$$

$$k_T = k_0 e^{\frac{-Ea}{RT}} \quad (6)$$

where C_0 is the initial anthocyanin content (AC) and C_t is the AC after t min of heating at a selected temperature. The kT is the kinetic constant at a given temperature, k_0 is the reaction rate constant (min^{-1}), R is the universal gas constant (8.314 J/mol.K), and T is the temperature (K).

Statistical analysis

Statistical analysis was performed with Minitab 19 software. Taguchi optimization results were analyzed by ANOVA. Significant extraction parameters in anthocyanin extraction were determined by F-value and corresponding p-value at a 95% confidence level. Statistical comparison of thermal degradation kinetic data was performed by Tukey's comparison test.

Results and Discussion

Taguchi optimization

In this study, the Taguchi optimization method was used to maximize TMA extraction. The average TMA results and the S/N ratios corresponding to each data are given in Table 2. According to the experimental results, the TMA content of the samples obtained by ultrasonic method ranged between 240.589 and 619.379 mg C3G/kg. In addition, the S/N ratios of the samples are between 47.63 dB and 55.84 dB. The results show that the extract with the highest TMA content was obtained when ultrasound was applied for 30 min to the mixture prepared in a 30:1 solvent: solute ratio in a solution containing 75% ethanol (A3B2C3). Similar results were obtained in the Taguchi L9 average S/N ratios table (Table 3). The highest value of each extraction parameter represents the

optimal level. The results revealed that the optimum extraction conditions were achieved by 10 min ultrasonic extraction of a sumac sample mixed with a solvent containing 75% ethanol at a solvent: solute ratio of 10:1 ($A_3B_1C_1$). The delta values in the table show the influence degree of the factors on the extraction process. Based on the data obtained, the order of influence of production factors on anthocyanin extraction is solvent: solute ratio (C) > ethanol concentration (A) > sonication time (B).

Table 3. Response table for S/N ratios

Level	A	B	C
1	51.20	*52.53*	*55.10*
2	51.87	51.59	52.62
3	*53.40*	52.35	48.76
Delta	2.21	0.94	6.34
Range	2	3	1

*italic characters indicate optimal levels of the process parameters.

The main effect graph of the S/N ratios for the Taguchi optimization method is provided in Figure 1. The graph indicates that the quantity of anthocyanins extracted from sumac increased as the concentration of ethanol in the solvent increased from 25% to 75%. Similar findings were obtained in anthocyanin extraction from jabuticaba peel (Rodrigues et al., 2015). The ANOVA table in Table 4 demonstrates that the effect of this extraction factor on the anthocyanin content was significant ($p < 0.05$). Another statistically significant effect was found for the solvent: solute ratio. The anthocyanin yield decreased with increasing solvent: solute ratio from 10:1 to 30:1 (Figure 1). Increasing solvent concentration in the extraction negatively affected anthocyanin diffusion from sumac powders. On the other hand, no significant effect of sonication time on anthocyanin extraction was observed (Figure 1). This effect was also not found statistically significant according to the ANOVA results in Table 4 ($p > 0.05$).

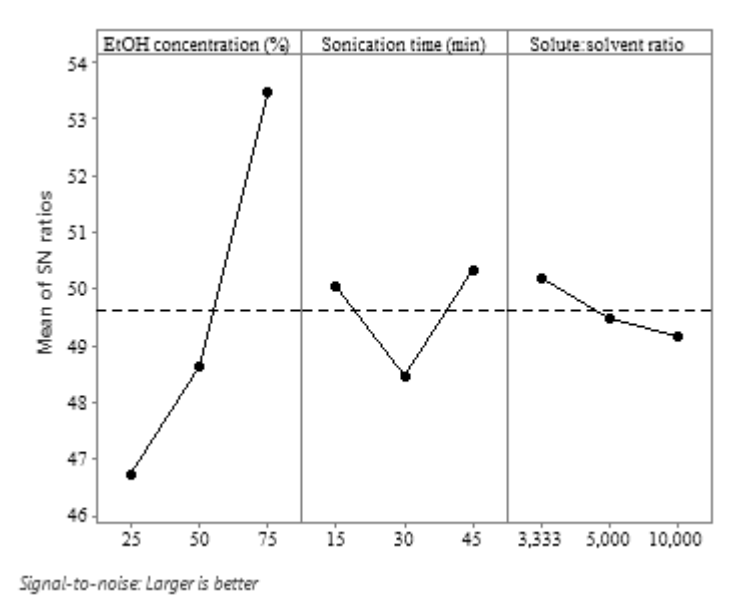

Figure 1. Main effects plot for S/N ratios

Table 4. ANOVA results for the extraction process of sumac anthocyanins

Source	DF	SS	MS	F-value	p-value
Ethanol concentration	2	16469	8234.3	23.90	0.040*
Sonication time	2	1411	705.7	2.05	0.328
Solvent: solute ratio	2	128988	64494.1	187.17	0.005*
Error	2	689	344.6		
Total	8	147557			

*significant; R^2= 99.53%, $R^2_{(adj)}$= 98.13%, $R^2_{(pred)}$=90.54%

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Validation tests were carried out to verify the optimum anthocyanin value obtained from the optimization process and to improve the anthocyanin extraction process. The anthocyanin content estimated by Minitab 19.0 software (630.071 mg C3G/kg) and the extraction yield obtained experimentally (632.024 mg C3G/kg) were found close to each other. Furthermore, an improvement of approximately 23% compared to the initial parameters ($A_1B_1C_1$) was achieved by applying Taguchi optimization.

Thermal stability of sumac anthocyanins

Thermal degradation of sumac anthocyanins was investigated at 70, 80 and 90 °C. The kinetic parameters of anthocyanin degradation by heat treatment are given in Table 5. It can be seen from Table 5 that the thermal degradation of sumac anthocyanins at each temperature level fits the first-order reaction model ($0.9945<R2<0.9986$). This result is in agreement with blackberry juice (Wang and Xu, 2007), blood orange juice (Kirca and Cemeroğlu, 2003), black carrot extract (Kirca et al., 2007), and wild blackthorn fruit extract (Moldovan et al., 2019) where a first-order reaction model for thermal degradation of anthocyanins was reported. The kinetic rate constant (k) is an indicator for predicting the thermal breakdown of color pigments. Lower k values indicate better color stability. The k values of sumac anthocyanins were determined as 1.69×10^{-3}, 2.65×10^{-3}, and 5.44×10^{-3} (min^{-1}) for 70, 80 and 90 °C, respectively (Table 5). The kinetic rate constant value of sumac anthocyanin extract increased significantly with increasing temperature from 70 °C to 90 °C ($p < 0.05$). This result indicates that anthocyanins subjected to heat treatment at 90 °C decomposed faster. The stability of sumac anthocyanins increased with decreasing half-life value ($t_{1/2}$). The $t_{1/2}$ value decreased significantly ($p < 0.05$) with increasing degree of heat treatment applied to the anthocyanin extract. Similar effects of kinetic rate constant (k) and half-life value ($t_{1/2}$) on anthocyanin stability were also reported for Juchara and Italian grape juices (Peron et al., 2017) and wild elm fruit extract (Moldovan et al., 2019). However, the activation energy (Ea) associated with the degradation of anthocyanins was 60.29 kJ/mol. Similar data were reported as 58.95 kJ/mol for blackberry juice (Wang and Xu, 2007), 62.5 kJ/mol for black carrot extract (Kirca et al., 2007), and 59.82 kJ/mol for purple sweet potato extract (Chen et al., 2019).

Table 5. Kinetic parameters of heat treatment stability of sumac anthocyanins

Temperature (°C)	$kx10^{-3}$ (min^{-1})	$t_{1/2}$ (hrs)	R^2	Ea (kJ/mol)
70	1.69^c	29.46^a	0.9945^a	60.28
80	2.65^b	18.75^b	0.9986^a	
90	5.44^a	9.15^c	0.9980^a	

a-c refers to the significant differences between the values in the same column (p <0.05).

Conclusion

In this study, ultrasound-assisted extraction of anthocyanins from sumac was successfully optimized using the Taguchi method. Under optimized parameter conditions, sumac anthocyanins were extracted with high extraction yields as a result of sonication with 75% ethanol-containing solvent at 10:1 solvent: solute ratio for 15 min. The decrease in the thermal stability of sumac anthocyanins with increasing temperature was proved by the first-order reaction model with increasing reaction rate constant (k) and decreasing half-life (t1/2) with increasing temperature. The thermal stability of sumac anthocyanins needs to be optimized considering food processing conditions.

Acknowledgments

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