

Determination of some physicochemical properties and anthocyanin extraction conditions of *Rosa damascena* Mill.

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Abstract

In this study, some physicochemical and bioactive properties of *Rosa damascena* Mill. and the effect of different extraction methods and conditions on total monomeric anthocyanin (TMA) extraction were investigated. The average petal weights, petal areas, L^* , a^* , and b^* values, dry matter, pH, titration acidity, L-ascorbic acid, TMA, and total phenolic content of the samples were 2.49 g, 6.04 cm², 54.01, 37.30, 22.72, 18.65%, 5.45, 0.11 mg/100 g, 1,366.52 mg/kg DW, 515.79 mg C3G Eq./100 g DM and 4,353.52 mg GAE/100 g DM, respectively. Catechin, ellagic acid, rutin, gallic acid, and two different anthocyanins were detected. The mass transfer coefficient values in conventional (20, 40, and 60°C) and ultrasound-assisted extraction (50%, 75%, and 100% amplitude) were 0.068, 0.324, and 0.922 ml/cm² and 2.178, 3.822, and 7.812 ml/cm² min, respectively. Results showed that anthocyanins can be extracted faster and with higher yields by ultrasound-assisted extraction.

Novelty impact statement

Rosa damascena Mill. petals have attractive red color and are rich sources of anthocyanins. Anthocyanins are water-soluble natural pigment and have the positive effects on human health. The anthocyanin extraction, which takes 210 min at 60°C with conventional extraction, can be shortened up to 18 min at 100% ultrasound amplitude by ultrasound-assisted extraction.

1 | INTRODUCTION

Rose plant (*Rosa* L.) is the most cultivated ornamental plant in the world with its pleasant scent and beautiful appearance. The genus *Rosa* has 200 species and more than 18,000 varieties (Khosh-Khui, 2014). The roses also have economic importance in terms of their petals, which are a source of natural fragrance, color, and flavoring (Baydar et al., 2004). It has been used traditionally in different food preparations. The rose petals have high antioxidant activity and are rich in bioactive substances such as ethereal oils, tannins, carotenoids, anthocyanins, organic acids, vitamins, and minerals (Butcaru et al., 2018). Anthocyanins are one of the most important polyphenol groups especially in red roses. The interest in foods containing anthocyanins have been increasing due to the positive effects on human health of anthocyanins (Tokgöz et al., 2010). Rose plant is a rich source of anthocyanins. Alizadeh and Fattahi (2021) reported

that the total anthocyanin content of *Rosa damascena* ranged from 0.5 to 3.5 mg cyanidin-3-glucoside/g DW. Anthocyanins have cardioprotective, anticancer, antidiabetic, antitumor, antimicrobial, and anti-inflammatory effects as a new source for functional food (Changxing et al., 2018; Krga & Milenkovic, 2019).

The rose plant is common in European and west Asia, especially in Iran, Afghanistan, Bulgaria, and Turkey (Skaria et al., 2007). Turkey is an important differentiation center in terms of rose diversity and has 70 different rose species (Özcelik et al., 2013). *R. damascena* Mill. is grown mainly in Iran, Bulgaria, and Turkey (Rasouli et al., 2018). It is used to make tea, jam, syrup, or sherbet in these regions. In this study, some physicochemical and bioactive properties of *R. damascena* Mill. were determined. In addition, it was aimed to determine the optimum extraction conditions of anthocyanins from rose petals by conventional and ultrasound-assisted extraction methods.

2 | MATERIALS AND METHODS

2.1 | Materials

R. damascena Mill. (Jam rose) was obtained from five different gardens in two different central districts [Edremit (E) and Şamranaltı (S)] in Van province. The samples were collected from the gardens with three replications. The botanical identification of the roses was made according to Flora of Turkey (Davis, 1985) by Prof. Dr. Şevket Alp at Department of Landscape Architecture in Van Yüzüncü Yıl University. Rose samples were immediately transported to laboratory by taking each rosebud into separate bags. Foreign substances were removed, the petals on the buds were separated and the petals were stored at -24°C until used in the analysis.

2.2 | Methods

2.2.1 | Determination of rosebud and petal weights of *R. damascena* Mill.

Rosebud weight of 10 roses randomly selected from the collected rose samples and the petal weight in rosebud were determined by weighing on the sensitive scale (Velioglu, 1990).

2.2.2 | Color measurement

L^* , a^* , and b^* values of the petals of roses were measured using CIE Lab Colorimeter (CSM 1, PCE Instruments, UK). C and h° values were calculated with the obtained color value (Anonymous, 1996).

2.2.3 | Petal area

After the petals were scanned on paper by scanner, the areas of the petals were measured on paper using an electronic planimeter (Koizumi Placom KP-90N, Nagaoka-shi, Japan; Caldas et al., 1992).

2.2.4 | Determination of some chemical parameters

Dry matter (DM), °brix, pH, and titration acidity was determined by the methods proposed by Association of Official Analytical Chemists (2003).

2.2.5 | L-ascorbic acid content

L-ascorbic acid was determined according to Lee and Coates (1999) with some modifications. Approximately 2 g of the sample and 5 ml of 4.5% metaphosphoric acid was homogenized in an ice bath with a homogenizer (30,000 rpm, 30 s). The homogenate was centrifuged

at $8,000\times g$ for 4 min at 4°C . The supernatant was filtered using a $0.45\text{ }\mu\text{m}$ polyvinylidene fluoride (PVDF) syringe filter and immediately injected into the HPLC system (Shimadzu, Kyoto, Japan) that consisted of a LC-20 AD gradient pump, a Rheodyne 7725i manual injection valve furnished with $20\text{ }\mu\text{l}$ of sample volume, a SPD-M20A diode array detector and CTO-10AS VP column oven. Separation of L-ascorbic acid was made in isocratic elution mode with a Waters Atlantis dC18 column ($4.6\times 250\text{ mm}$, $5\text{ }\mu\text{m}$) at 25°C . Purified water acidified with H_2SO_4 (pH 2.54) was used as the mobile phase with a flow rate of 0.7 ml/min . The measurement was made at 244 nm. L-ascorbic acid in the chromatogram was identified base on its retention time and spectral data by comparison with the L-ascorbic acid standard (mg/kg DM).

2.2.6 | Methanolic extracts of rose petals

The rose petals (2.5 g) and 20 ml of acidified methanol (1% HCl) in a test tube were kept in an ultrasonic bath for 15 min. The tube was centrifuged at $6,000\times g$ at 4°C for 10 min. The supernatant was removed and the same procedure was repeated twice more with the remaining pulp. The collected supernatant was dried with a rotary evaporator. The residue was dissolved in 10 ml of methanol containing 1% HCl. Methanolic extracts were stored at -24°C until further analyses (Lončarić et al., 2016).

2.2.7 | Total phenolic content and total flavonoid content

Total phenolic content (TPC) was determined by the Folin–Ciocalteu method (Singleton & Rossi, 1965). The results were expressed as gallic acid equivalent (mg GA Eq./kg DM). Total flavonoid content (TFC) was determined by the AlCl_3 method (Zhishen et al., 1999). The results are expressed as quercetin equivalent (mg Qu Eq./g DM).

2.2.8 | Phenolic content and anthocyanin distribution

Analyses of the phenolics and anthocyanins were carried out using the HPLC system (Shimadzu, Kyoto, Japan; Colaric et al., 2005). Separation of phenolic substances was performed with a Waters Symetry C18 column ($250\times 4.6\text{ mm}$ id, particle size $5\text{ }\mu\text{m}$) at 25°C . Solvents were 2% acetic acid (A) and 5% acetic acid water: acetonitrile (1:1) (B). Gradient program was as follows: 90% A at 0 min, 80% A at 30 min, and 55% A at 60 min at a flow rate of 1 ml/min . The wavelength was determined as 280 nm for benzoic acid derivatives and flavan 3-ols, 360 nm for flavonols and ellagic acid, and 520 nm for anthocyanins. For determination of the anthocyanin peaks seen in the HPLC chromatogram, the eluents of the peak were collected separately at the detector exit and then injected in the LC-MS/MS (Thermo Scientific, Q Exactive) device. Samples

were injected into LC-MS/MS coupled precision-mass (HR/AM) orbitrap detector and Waters Symetry C18 (250 × 4.6 mm id, particle size 5 µm) column at 25°C. The LC gradient followed a linear gradient elution program with 2% acetic acid–water (A), 0.5% acetic acid water: acetonitrile (1:1) (B) at a flow rate of 8.0 µl/s. Gradient program was as follows: 10% B at 0 min, 10% B at 4 min, 90% B at 24 min, 10% B at 25 min. The wavelength of the UV-visible detector is 520 nm. Mass spectrometry (MS) parameters are as follows: Nebulizer pressure 45 psi, spray voltage 3,000 V, capillary temperature 350°C, carrier gas temperature 280°C, carrier gas flow rate 10 L/min. The device was operated in positive ion mode from m/z^+ 100 to 1,000 with electrospray ionization scanning, collision gas pressure 50% and collision energy 50%. The results were expressed as cyanidin-3-O-glucoside equivalent (mg C3G Eq./kg DM).

2.2.9 | Antioxidant activity analysis (DPPH and ABTS)

DPPH assay was performed according to Pyo et al. (2004), and the results were expressed as Trolox equivalent antioxidant capacity (mmol Trolox Eq./g DM). The ABTS assay was performed according to the method described by Re et al. (1999). The results were expressed as Trolox equivalent antioxidant capacity (mmol Trolox Eq./g DM).

2.2.10 | Total monomeric anthocyanin content

Total monomeric anthocyanin (TMA) content was determined by the pH difference method (Lee et al., 2005). For this purpose, two different buffers with pH 1 and pH 4.5 were prepared. The both buffers were added on methanolic extracts into a separated test tube, and absorbance was measured at 528 and 700 nm. TMA content was calculated with the following equations.

$$\text{Absorbance difference (A)} = (A_{528} - A_{700})_{\text{pH } 1.0} - (A_{528} - A_{700})_{\text{pH } 4.5}$$

$$\text{TMA, mg/L} = (A \times \text{MW} \times \text{DF} \times 1,000) / (\epsilon \times l)$$

A = absorption, MW = molecular weight, DF = dilution factor, and ϵ = molar absorption. The results were determined as cyanidin-3-glucoside (C3G) equivalent.

2.2.11 | Anthocyanin extraction from *R. damascena* Mill.

R. damascena Mill. petals were subjected to two different extraction techniques (conventional and ultrasound-assisted extraction) for anthocyanin extraction. In all extraction applications, petals (10 g) were placed in the jar (380 ml) with 150 ml of water: citric

acid solution (99:1, w:v). Petal/solvent ratio was determined through preliminary experiments.

Conventional extraction

The conventional extraction was carried out at three different temperatures (20, 40, and 60°C) in a shaking water bath (WSB-30, Daihan, Korea) at 125 rpm. Conventional extraction at 20, 40, and 60°C was terminated at 2,880th, 420th, and 360th minutes, respectively. During the extraction, samples were taken at certain intervals. To determine the effect of the acidity on anthocyanin extraction, the samples were extracted with 0.75%, 1%, and 1.25% citric acid solution at 60°C.

Ultrasound-assisted extraction

The ultrasound-assisted extraction was performed using a Bandelein Sonoplus HD 3200 ultrasonic homogenizer (Germany). The ultrasonic probe used was Bandelin UW 3200 (200 W max. power, 20 kHz, Germany). The samples were sonicated at 39 ± 2 , 41 ± 2 , and 50 ± 2 °C at 50%, 75%, and 100% amplitudes of output power, respectively. Ultrasound-assisted extraction was terminated at the 100th, 50th, and 30th minutes, respectively. For TMA analysis, samples were taken at certain intervals during the extraction.

Calculation of mass transfer coefficient (K_L)

The study focused on the determination of the final stage of the extraction and samples were taken at fixed time intervals. In the several mathematical models such as Peleg, rate law, Fick's model, etc., samples should be taken very often at the beginning of the extraction. Therefore, these models cannot be applied to our experimental data. For the comparison of extraction conditions, mass transfer coefficient (K_L) value calculated from the mass flow equation of Heldman and Singh (1981) was used.

$$C = C_s \{ 1 - \exp [- (K_L \times A) / (V \times t)] \}$$

The values of K_L = mass transfer coefficient, C = the concentration of the soluble substance in solvent at any time, C_s = the concentration of the soluble substance in solvent in a saturated state, t = time (min), A = surface area (cm²), and V = the volume of the solvent (160 ml).

The C value at the initial time (t = 0) was assumed to be zero. Calculated $\ln(1 - C/C_s)$ values versus the times were plotted for each sample. The slopes were equal to the " $K_L \times A/V$ " and K_L (ml/cm² min) was calculated in the equation.

2.2.12 | Statistical evaluation

The differences between samples and applications were determined by one-way analysis of variance (one-way ANOVA) using the SPSS Statistic 20 computer program. Duncan's multiple comparison test was used to evaluate the differences between the means.

3 | RESULTS AND DISCUSSION

3.1 | Rosebud weights, petal weights, and petal area of *R. damascena* Mill.

Some physical properties of *R. damascena* Mill. were given in Table 1. Petal area, rosebud, and petal weight of *R. damascena* Mill. ranged from 5.87 to 6.18 cm², 4.24 to 5.21 g, and 2.15 to 2.75 g, respectively. Although the differences among gardens in terms of rosebud and petal weight were found to be significant ($p < .05$), the differences for petal areas were found to be insignificant ($p > .05$). There is a positive linear relationship between rosebud and petal weight ($r = .652$, $p < .05$). E2 sample has the highest rosebud and petal weight. Velioglu (1990) reported that the rosebud and petal weight of *R. damascena* from Isparta Province was 2.14 and 1.58 g, respectively. Rosebud and petal weights of *R. damascena* plants from different regions of Iran ranged from 1.0 to 2.4 g and 0.9 to 1.3 g, respectively (Zeinali et al., 2009). Our results are in the range given for rosebud, but the petal weight is higher than the reported values. This may be due to the effects of differences in species, geographical conditions, climate, and growing conditions on physical properties.

The main factor affecting plant color is genetic factors, as well as environmental factors such as climate, soil quality, maturity level, cultivation technique, stress, etc. (Şener, 2012). L^* , a^* , b^* , C , and h° values of the petals of *R. damascena* Mill. plants were also determined (Table 1). The L^* (lightness), $+a^*$ (redness) and $-b^*$ (blueness) varied between 47.79 and 65.06, 26.79 and 41.44, (−46.20) and (−9.88), respectively. E1 sample had the highest $-b^*$ and E2 sample had the highest $+a^*$ values. The intensity of the blue and red colors is due to the presence of anthocyanins. Chroma (C) value, which is an indicator of saturation in color, was ranged from 28.56 to 60.83. The h° value changing from 270° to 360° is an indicator of a color transition from blue to red. The highest h° value was calculated as 339.72 in the E3 sample, and the lowest was 315.85 in the E1 sample. It can be said that *R. damascena* Mill. plants are much closer to the red color. The difference between gardens in terms of all color parameters (L^* , a^* , b^* , C , and h°) of *R. damascena* Mill. plant is significant ($p < .05$). Şengül et al. (2017) noted that the L^* , a^* , and b^* values of *R. damascena* Mill. from Isparta province were 38.98, 17.30, and −3.44, respectively. The reported values by Şengül et al. (2017) are quite lower than our results. The L^* , a^* , and b^* values of the petals of *R. damascena* grown in India were reported as 53.54, 17.25, and −0.6, respectively (Kumar et al., 2017). Although the L^* value was within range of our values, a^* value was found to be lower than our values and the b^* value was found to be quite higher.

3.2 | Some chemical properties of *R. damascena* Mill.

The DM, °brix, pH, and titratable acidity content of the petals of the *R. damascena* Mill. plant was given in Table 2. DM, °Bx, pH, and acidity contents of sample ranged from 17.40% to 19.43%, 11.00%

TABLE 1 Some physical properties of *Rosa damascena* Mill.

Samples	Color parameter values						
	Rosebud weight (g)	Petal weight (g)	Petal area (cm ²)	L^*	a^*	b^*	h°
E1	4.24 ± 0.60 ^a	2.49 ± 0.50 ^{ab}	5.87 ± 0.41 ^a	47.79 ± 0.45 ^a	39.52 ± 3.26 ^{ab}	−46.20 ± 6.21 ^a	315.85 ± 3.47 ^a
E2	5.21 ± 0.85 ^b	2.75 ± 0.43 ^b	6.11 ± 0.86 ^a	51.28 ± 4.74 ^{ab}	41.44 ± 3.64 ^c	−20.18 ± 4.50 ^b	334.14 ± 4.37 ^b
E3	4.64 ± 0.62 ^{ab}	2.15 ± 0.08 ^a	6.18 ± 0.23 ^a	65.06 ± 3.76 ^d	26.79 ± 3.36 ^a	−9.88 ± 1.59 ^c	339.72 ± 1.76 ^c
S1	5.07 ± 0.62 ^{ab}	2.66 ± 0.33 ^{ab}	5.92 ± 0.31 ^a	54.72 ± 4.67 ^c	37.80 ± 3.98 ^b	−18.94 ± 2.92 ^b	333.66 ± 3.69 ^b
S2	4.56 ± 0.80 ^{ab}	2.40 ± 0.36 ^{ab}	6.12 ± 0.23 ^a	51.18 ± 4.97 ^{ab}	40.94 ± 3.89 ^c	−18.40 ± 1.89 ^b	335.27 ± 3.40 ^b
Mean ± SD	4.74 ± 0.69	2.49 ± 0.34	6.04 ± 0.41	54.01 ± 3.72	37.30 ± 3.63	−22.72 ± 3.42	331.73 ± 3.34

Note: Values are given as mean ± SD. The differences between the means shown with different letters in the same column are statistically significant ($p < .05$).

TABLE 2 Chemical properties of *Rosa damascena* Mill.

Samples	Dry matter (%)	°Brix	pH	Titration acidity (mg/100 g)	Total monomeric anthocyanin (mg C3G Eq./100 g dry matter [DM])	Total phenolic content (mg GAE/100 g DM)	Total flavonoid content (mg Qu Eq./g DM)	Antioxidant activity (mmol Trol. Eq./g DM)	
								DPPH	ABTS
E1	19.43 ± 0.24 ^a	11.20 ± 0.14 ^a	5.35 ± 0.07 ^b	0.112 ± 0.012 ^a	507.23 ± 5.90 ^b	3,777.84 ± 230.61 ^a	76.56 ± 1.52 ^a	1,642.02 ± 179.01 ^a	379.44 ± 5.02 ^a
E2	18.91 ± 0.73 ^a	11.05 ± 0.07 ^a	5.21 ± 0.02 ^a	0.117 ± 0.015 ^a	594.90 ± 0.00 ^c	4,886.93 ± 378.46 ^b	95.19 ± 6.23 ^{ab}	1,687.22 ± 249.33 ^a	381.21 ± 1.26 ^{ab}
E3	18.85 ± 0.64 ^a	11.05 ± 0.07 ^a	5.65 ± 0.01 ^c	0.104 ± 0.011 ^a	403.90 ± 16.24 ^a	3,747.16 ± 306.14 ^a	98.81 ± 1.56 ^b	1,542.56 ± 268.51 ^a	374.55 ± 1.88 ^a
S1	17.40 ± 0.02 ^b	11.00 ± 0.14 ^a	5.40 ± 0.04 ^b	0.111 ± 0.012 ^a	476.96 ± 31.00 ^b	4,017.61 ± 368.82 ^a	83.15 ± 5.08 ^c	1,691.74 ± 134.26 ^a	389.20 ± 1.26 ^b
S2	18.66 ± 0.07 ^a	11.15 ± 0.07 ^a	5.64 ± 0.02 ^c	0.108 ± 0.014 ^a	595.94 ± 19.19 ^c	5,338.07 ± 339.89 ^b	72.09 ± 3.17 ^c	1,721.13 ± 105.49 ^a	411.39 ± 5.02 ^c
Mean ± SD	18.65 ± 0.34	11.11 ± 0.10	5.45 ± 0.03	0.11 ± 0.013	515.79 ± 14.47	4,353.52 ± 324.78	85.16 ± 3.51	1,656.93 ± 187.32	387.16 ± 2.89

Note: Values are given as mean ± SD. The differences between the means shown with different letters in the same column are statistically significant ($p < .05$).

to 11.20%, 5.21 to 5.64 and 0.104 to 0.117 mg/100 g, respectively. The differences among gardens for only DM and pH was found to be significant ($p < .05$). Although the highest DM, and °Bx values were determined in the E1 sample, the highest pH and lowest acidity were also found in the E3 samples. The DM and °Bx values were similar to the data reported by Şengül et al. (2017) (18.43% DM, %10.35 °Bx) and Velioglu (1990) (19.32% DM, %11 °Bx), respectively. Kumar et al. (2017) and Şengül et al. (2017) reported the pH value of fresh petals as 5.47 and 5.30, respectively. These values are within the range of values reported in the study.

3.3 | TPC, TFC, total monomeric anthocyanin contents, and antioxidant activity of *R. damascena* Mill.

The TPC, TFC, and TMA content of samples was given in Table 2. The TPC, TFC, and TMA content of *R. damascena* Mill. petals were found in the range of 403.90–595.94 mg C3G Eq./100 g DM, 3747.16–5338.07 mg GAE/100 g DM, and 72.09–98.81 mg Qu Eq./g DM, respectively. For TMA and TFC content, the differences between gardens were statistically significant ($p < .05$). While S2 sample had the highest TMA content, E3 had the highest TFC content. It was determined that there is a positive linear relationship between TMA and a^* value ($r = .782$, $p < .01$). This relationship is due to the red color of the sample.

The TPC of the *R. damascena* was found as 308 mg GAE/100 g DM and 110.07 µg GAE/mg by Kalcheva-Karadzhova et al. (2014) and Sivaraj et al. (2019), respectively. For TPC, while our result was lower than the value reported by Sivaraj et al. (2019), higher than those of Kalcheva-Karadzhova et al. (2014). The TFC of the *R. damascena* plant grown in Iran was found as 1,240 mg Qu/100 g by Rezvani-Kamran et al. (2017). Sivaraj et al. (2019) reported that the TFC of the extracts obtained from *R. damascena* grown in India was 176.00 µg Qu/mg. The TFC values in our study are lower than the reported values.

Kumar et al. (2017) and Kalcheva-Karadzhova et al. (2014) reported that the total anthocyanin concentration of fresh *R. damascena* plant was 98.64 mg/100 g and 12.6 mg C3G Eq./100 g, respectively. Our results were higher than reported value in the literature. The fluctuations in temperature and excessive or insufficient precipitation negatively affect anthocyanin contents of rose plants (Şengül et al., 2017). These results may indicate the suitability of factors such as temperature and precipitation in the region for anthocyanin accumulation in *R. damascena* Mill.

The DPPH and ABTS content of samples ranged from 1,542.56 to 1,721.13 and 374.55 to 411.39 mmol Trol. Eq./g DM, respectively (Table 2). The highest DPPH and ABTS values were determined in the S2 sample that have the highest TPC and TMA levels. However, no significant correlation was found between the TMA and DPPH, TMA, and ABTS ($p > .05$). Damar (2010) reported that no significant correlation was found between antioxidant capacity and TMA in cherry juice ($p > .05$). The DPPH value of *R. damascena* Mill.

grown in Bulgaria was reported as 2,163 $\mu\text{mol Trol. Eq.}/100 \text{ g DM}$ by Kalcheva-Karadzova et al. (2014). The reported value is higher than our results. For all analyzed parameters, the differences may be due to differences in the variety, environmental conditions, cultivation technique, and maturity level.

3.4 | L-ascorbic acid, phenolic and anthocyanin compounds of *R. damascena* Mill.

The L-ascorbic acid contents of *R. damascena* Mill. samples were given in Table 3. The L-ascorbic acid content of the petals varied between 943.53 and 1,740.48 mg/kg DM. As in TPC and TMA, the highest L-ascorbic acid content was determined in the S2 sample. However, the differences among gardens were not found to be significant ($p > .05$). The L-ascorbic acid content of fresh *R. damascena* petals was found as 45.00, 70.47 and 293.37 mg/100 g by Şengül et al. (2017), Prata et al. (2017) and Kumar et al. (2017), respectively. On a DM basis, the result reported by Kumar et al. (2017) are higher than our results. However, our results were higher than the findings of the other researchers.

Gallic acid, catechin, ellagic acid, and rutin content of the *R. damascena* Mill. samples were 19.57–48.66, 137.64–279.33, 103.27–436.38 and 1,155.97–2,501.34 mg/kg DM, respectively (Table 3). E3 sample had the highest catechin content, S1 had the highest rutin, and S2 had the highest gallic and ellagic acid content. The amount of ellagic acid in samples from Şamranaltı region was significantly higher than that of the samples from the Edremit. There is also a positive linear relationship between ABTS and ellagic acid content ($r = .806$, $p < .01$). It may be due to the ABTS analysis better reflecting the antioxidant effect of ellagic acid. For individual phenolic compounds, the differences between the samples collected from different regions and gardens were found to be statistically significant ($p < .05$). The catechin content of *R. damascena* Mill. was 3.1 mg/g DM (Kalcheva-Karadzova et al., 2014). Reported value are higher than our results. The ellagic acid content of the *Rosa rugosa* plant ranged from 47.30 to 50.90 mg/100 g wet weight (Cendrowski et al., 2017). On a DM basis, ellagic acid contents of S1 and S2 samples were similar to the reported value by Cendrowski et al. (2017). The gallic acid content of fresh Taif roses, a variety of *R. damascena* Mill., was 10.01 mg/g (Abdel-Hameed et al., 2016). The results in this study for gallic acid were lower than the reported value. The amount of L-ascorbic acid and individual phenolics in rose samples varies according to the variety, altitude, ecological conditions, harvest time, and postharvest technology (Ercişi, 1996).

In the study, two anthocyanin peaks (Anthocyanin 1 and 2) on chromatograms of sample extracts were determined by HPLC. When the obtained peaks were detected in LC-MS/MS, anthocyanin-1 and 2 peaks gave positive ionization with H^+ and Na^+ , respectively. Chromatographic, spectroscopic, and mass spectral properties of anthocyanins detected in rose petals are given in Table 4. The both peaks have the same spectrum (λ_{max}) as 277–512 nm. The spectrum (λ_{max}) of cyanidin-3,5-diglucoside

TABLE 3 L-ascorbic acid, phenolics, and anthocyanins of *Rosa damascena* Mill.

Samples	L-ascorbic acid (mg/kg DM)	Gallic acid (mg/kg DM)	Catechin (mg/kg DM)	Ellagic acid (mg/kg DM)	Rutin (mg/kg DM)	Anthocyanins (mg C3G Eq./kg DM)	
						Anthocyanin-1	Anthocyanin-2
E1	943.53 $\pm$ 59.56 ^a	19.57 $\pm$ 5.34 ^a	198.76 $\pm$ 86.06 ^{ab}	103.27 $\pm$ 13.41 ^a	2,389.19 $\pm$ 436.03 ^b	2,927.54 $\pm$ 38.2 ^e	3,036.85 $\pm$ 0.96 ^d
E2	1,234.22 $\pm$ 18.46 ^a	20.60 $\pm$ 2.68 ^a	174.09 $\pm$ 29.46 ^{ab}	137.20 $\pm$ 29.27 ^a	1,155.97 $\pm$ 121.13 ^a	2,744.59 $\pm$ 72.20 ^d	2,873.87 $\pm$ 53.94 ^d
E3	1,392.08 $\pm$ 30.38 ^a	46.85 $\pm$ 4.44 ^b	279.33 $\pm$ 13.94 ^b	125.28 $\pm$ 6.94 ^a	2,256.56 $\pm$ 140.33 ^b	2,313.61 $\pm$ 69.99 ^c	2,524.63 $\pm$ 26.65 ^c
S1	1,513.28 $\pm$ 36.46 ^a	48.36 $\pm$ 2.57 ^b	241.30 $\pm$ 37.11 ^{ab}	415.96 $\pm$ 59.78 ^b	2,501.34 $\pm$ 0.36 ^b	1,040.90 $\pm$ 56.85 ^a	1,059.98 $\pm$ 12.50 ^a
S2	1,749.48 $\pm$ 79.32 ^a	48.66 $\pm$ 0.37 ^b	137.64 $\pm$ 30.39 ^a	436.38 $\pm$ 50.47 ^b	2,324.18 $\pm$ 245.32 ^b	1,530.47 $\pm$ 40.41 ^b	1,724.51 $\pm$ 8.93 ^b
Mean $\pm$ SD	1,366.52 $\pm$ 44.84	36.81 $\pm$ 4.28	206.22 $\pm$ 39.39	243.62 $\pm$ 31.97	2,125.45 $\pm$ 188.63	2,111.42 $\pm$ 55.54	2,243.97 $\pm$ 20.60

Note: Values are given as mean $\pm$ SD. The differences between the means shown with different letters in the same column are statistically significant ($p < .05$).

TABLE 4 Chromatographic, spectroscopic, and mass spectral properties of anthocyanins detected in petals

Peaks	Rt (min)	λ_{max} (nm)	Molecular ion precursor (m/z)	$[M]^+$	Molecular formula
Anthocyanin-1	17.5	277–512	612.16 $[M+H]^+$	611.16	$C_{27}H_{31}O_{16}$
Anthocyanin-2	26	277–512	634.15 $[M+Na]^+$	611.16	$C_{27}H_{31}O_{16}$

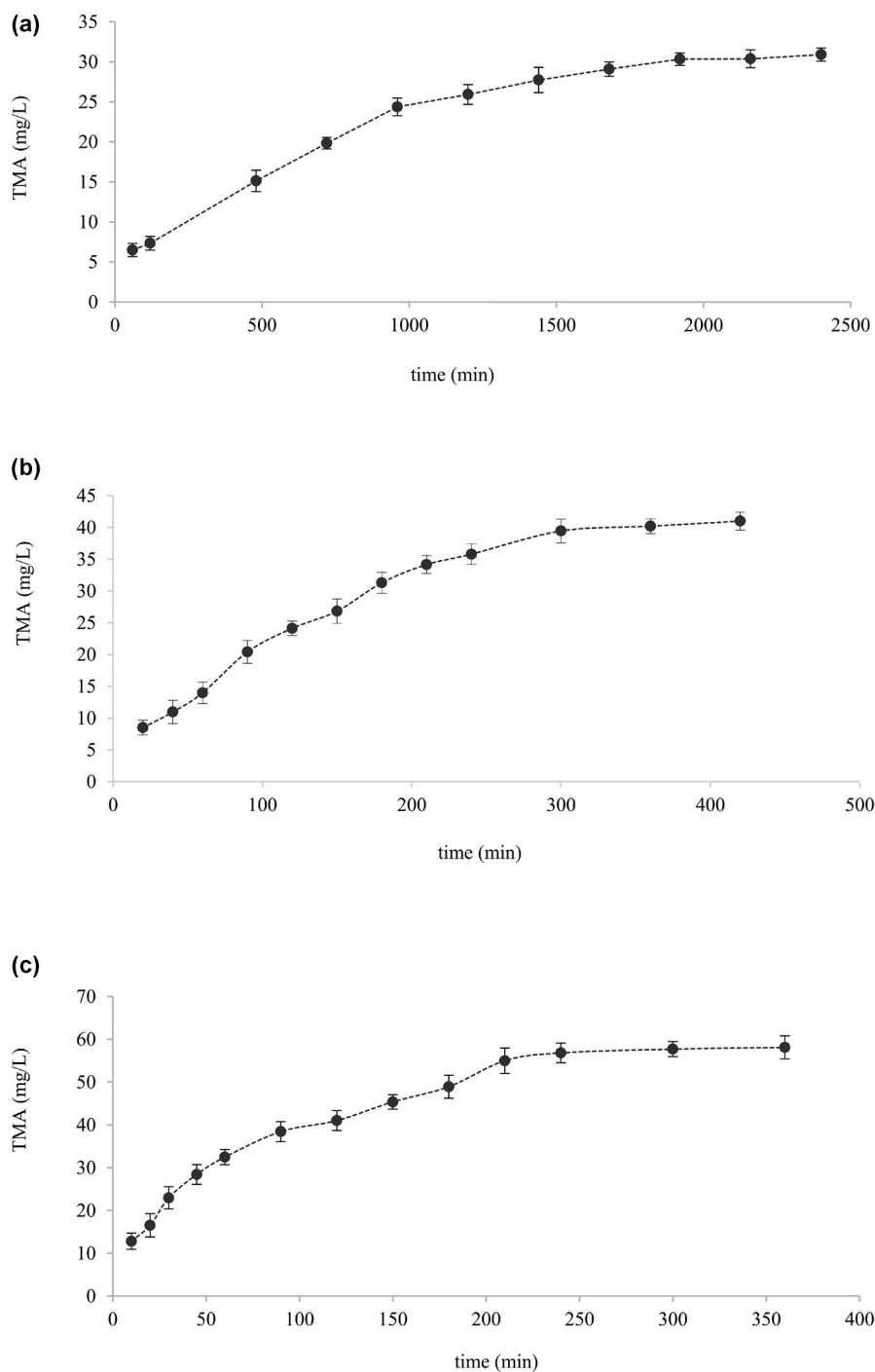


FIGURE 1 Total monomeric anthocyanin transition at different temperatures during conventional extraction (a = 20°C, b = 40°C, c = 60°C)

anthocyanin in *Rosa* spp. is reported as 277.2–512.6 nm by Wan et al. (2019). Molecular weights $[M]^+$ (m/z) of anthocyanins 1 and 2 were determined as 611. In the *Dioscorea alata* (purple

yam) plant, the $[M]^+$ (m/z) values of cyanidin-3,5-diglucoside and cyanidin-3-diglucoside isomers with the same closed formulas ($C_{27}H_{31}O_{16}$) were found as 611 (Srivichai & Hongsprabhas, 2020).

Jia et al. (2008) reported that in *Herbaceous peony*, the value of $[M]^+ (m/z)$ of cyanidin-3-O-glucoside-5-O-galactoside ($C_{27}H_{31}O_{16}$) was 611. Anthocyanin-1 and 2 in this study have the same UV spectra and molecular weight (Table 4). These two anthocyanins may be different cyanidin isomers containing two molecules of glucose with the same closed formulas such as cyanidin-3,5-diglucoside, cyanidin-3-diglucoside, or cyanidin-3-O-glucoside-5-O-galactoside. The amounts and types of anthocyanins in rose plants vary according to the temperature and pH value of the environment, precipitation and irrigation (Şengül et al., 2017). The average amount of anthocyanin-1 and 2 in the samples was

determined as 2,111.42 and 2,243.97 mg C3G Eq./kg DM, respectively (Table 3). E1 had the highest anthocyanin-1 and 2 contents.

3.5 | Extraction of total anthocyanin by ultrasound-assisted and conventional methods

In the extraction studies, petals were subjected to conventional and ultrasound-assisted extraction with different parameters. The results are given in Figures 1–3. In conventional extraction, TMA content increased with increasing temperature, acidity and time.

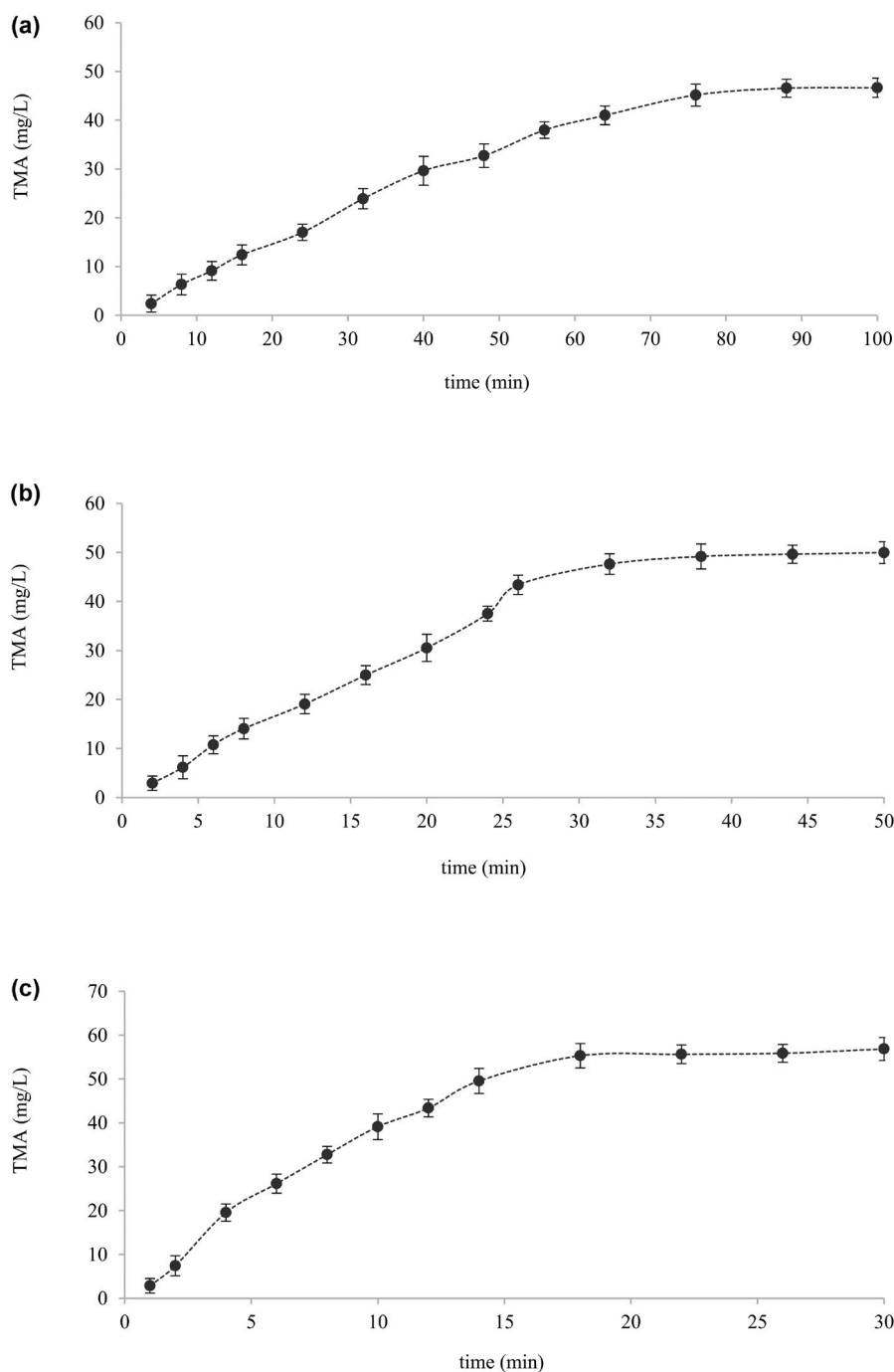


FIGURE 2 Total monomeric anthocyanin transition of different amplitudes during ultrasonic extraction (a = 50%, b = 75%, c = 100%)

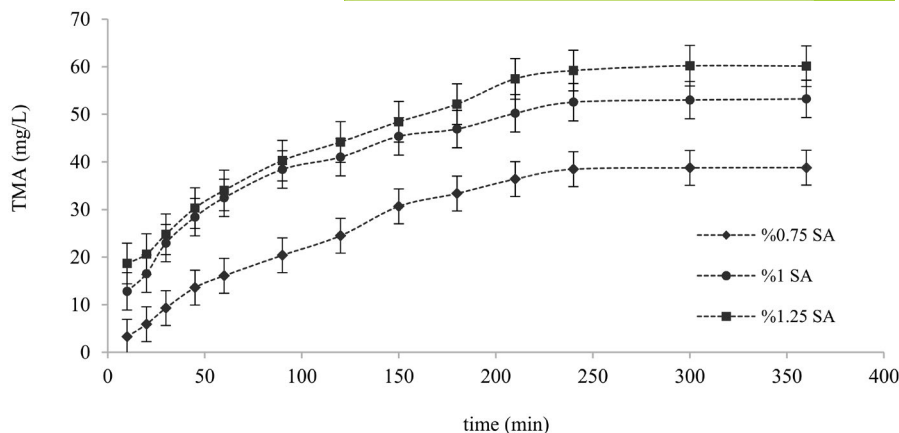


FIGURE 3 Total monomeric anthocyanin transition due to different acid ratios in the solvent at 60°C during conventional extraction

TABLE 5 Mass transfer coefficients of anthocyanin extraction with different extraction methods and conditions (ml/cm² min)

Conventional extraction			
Temperature (°C)	Time (hr)	Solvent acidity level	Mass transfer coefficient (Mean ± SD)
20	48	%1 SA	0.068 ± 0.004 ^a
40	7	%1 SA	0.324 ± 0.004 ^b
60	6	%0.75 SA	0.730 ± 0.042 ^A
		%1 SA	0.922 ± 0.015 ^{cAB}
		%1.25 SA	1.092 ± 0.066 ^B
Ultrasound-assisted extraction			
Amplitude	Time (min)	Solvent acidity level	Mass transfer coefficient (Mean ± SD)
%50	100	%1 SA	2.178 ± 0.013 ^a
%75	50	%1 SA	3.822 ± 0.044 ^b
%100	30	%1 SA	7.812 ± 0.011 ^c

Note: Values are given as mean ± SD. The difference between temperature and ultrasonic amplitude applications is given in the same column in lower case letters. The difference between acidity applications is given in the same column in capital letters for only 60°C ($p < .05$).

The highest TMA contents in conventional extraction were found at 60°C in 1.25% citric acid solution. In the ultrasound-assisted extraction, TMA content increased with increasing ultrasound amplitude and duration of ultrasound treatment. With reaching the saturation point in the extraction, the slope started to decrease. Therefore, the extraction can be terminated from the point where the change becomes insignificant. Conventional extraction can be cut in 210th min at 60°C, 300th minute at 40°C, and 1,680th min at 20°C. Extraction can be terminated in the 18th min at 100% amplitude, the 32th min at 75% amplitude, and the 76th min at 50% amplitude in ultrasound-assisted extraction. After conventional extraction in 210th min at 60°C and ultrasound-assisted extraction in the 18th min at 100% amplitude, total anthocyanin contents of sample extracts were 54.98 and 55.30 mg/L, respectively. Results show that ultrasound-assisted extraction is much faster and more effective than conventional extraction.

Extraction flow rate is a measure of the transfer of soluble components from one phase to the other within a specified period of time. Mass transfer coefficient is used to calculate the extraction flow rate and compare the efficiency of extraction. The K_L values of the extractions are given in Table 5. K_L values for conventional extraction ranged from 0.068 to 1.092 ml/cm² min. The increasing temperature and solvent acidity increased the K_L value of TMA. There are differences among K_L values for solvent acidity (0.75%, 1, and 1.25), but the differences were not statistically significant ($p > .05$). On the other hand, the difference between temperatures was found to be statistically significant ($p < .05$). The K_L values calculated for ultrasound-assisted extractions varied between 2.178 and 7.812 ml/cm² min. The differences among the amplitude applications (50%, 75%, and 100%) were statistically significant ($p < .05$). Results show that mass transfer coefficient of ultrasound-assisted extraction in the 18th min at 100% amplitude is around eight times

higher than those of conventional extraction in 210th min at 60°C. In solid-liquid extraction, the diffusion coefficient of the solvent in the particle and components in the solvent is expected to increase with temperature, and thus the K_L in extraction increases. The K_L in solid-liquid extractions depends on many other factors such as solvent type, particle size, and mixing. The application of ultrasound causes the rapid diffusion of the solvent into the cellular material due to the breakdown in the cell wall. As a result, the K_L value increases depending on the increasing mass transfer rate (Cavuldak et al., 2016; Şengül & Topdaş, 2019). The effect of temperature (20, 50, and 70°C) on water-soluble content extraction from dried mulberry with water was investigated by Bakkalbaşı (2001). The K_L values was found as 2.91 ml/min at 20°C, 4.68 ml/min at 50°C, and 5.52 ml/min at 70°C.

4 | CONCLUSIONS

In this study, some physicochemical and bioactive properties of the *R. damascena* Mill. plants were determined. In addition, anthocyanin extraction from *R. damascena* Mill. with different methods and conditions was investigated. *R. damascena* Mill. plant is a rich natural source of bioactive compounds (L-ascorbic acid, phenolic compounds, and anthocyanins). This study draws attention to a natural colorant aspect of *R. damascena* Mill. with its high anthocyanin content. It was determined that the increase in acidity, temperature, and ultrasonic amplitude in the extraction applications increased the mass transfer coefficient (K_L) of the TMA. As a result, the study showed that in the extraction of anthocyanins from rose petals, ultrasound-assisted extraction is more rapid and effective than conventional extraction. The anthocyanin extraction process, which takes 210 min at 60°C with conventional extraction, can be reduced to 18 min with ultrasound-assisted extraction.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

Gülşen Berat Torusdağ: Data curation; Formal analysis; Investigation; Methodology; Visualization; Writing – original draft. **Emre Bakkalbaşı:** Conceptualization; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing – review & editing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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