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The Usability of Crab Apple (*Malus floribunda*) Anthocyanins as a Natural Colorant in Apple Marmalade

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HIGHLIGHTS

- Crab apple anthocyanins can be used as a natural colorant source instead of synthetic colorants
- Crab apple phenolics have added functional properties to apple marmalade.
- Antioxidant activity and *a** value decreased as the storage temperature and time increased in apple marmalade.

Abstract: In this study, the usability of crab apple as a natural food colorant for apple marmalade was investigated. Marmalades were stored at 9, 22, and 35 ºC for 6 months and analyzed some physicochemical and biochemical parameters. Titratable acidity decreased with the increase of storage temperature and time in marmalades compared to the initial values. As the storage temperature and time increased, the lightness (*L**), redness (*a**), chroma (C*) values of the samples decreased, while the hue angle (*h*) and yellowness (*b**) values increased. The increase in temperature and time during the storage period caused a significant decrease in total phenolic content, total monomeric anthocyanin, and antioxidant activity values. The degradation of anthocyanins during storage occurred according to first-order reaction kinetics. According to the results obtained, the addition of crab apple juice concentrate allowed the desired level of color to be formed in apple marmalade as well as a functional product.

Keywords: anthocyanin; crab apple; marmalade; natural food colorant; storage.

INTRODUCTION

In addition to being an important sensory feature in determining the quality of foods, colour is also effective in determining consumer preferences [1]. Therefore, it is important to minimize the loss of pigment that may occur during the production and storage of foods [2]. In the food industry, the typical natural color

appearance of food or beverages gives a high quality, while an artificial glossy appearance gives the opposite impression [3]. The color of fruits and vegetables changes depending on the food production and storage process, as well as seasonal changes, species differences, soil conditions and post-harvest practices [4]. Synthetic colorants are used as food additives after produced by chemical synthesis and determined safe use conditions. In addition, it has important advantages such as ease of production, high purity, strong color, color ranges, heat, light and pH stability, not causing taste-odor changes and low cost. However, technological developments and different trends in consumer expectations have increased the demand for natural colorants [5-7]. Natural colorants are organic structures obtained from edible natural sources by various methods [6] and found in different plant parts such as leaves, flowers, and fruits [8,9]. One of the main groups of natural food colorants is red-violet anthocyanins and an important alternative in functional food production instead of synthetic colorants [1,4,10]. There are many fruits and vegetables (black carrot, blueberry, red radish, purple sweet potato, cherry, black mulberry and black grape, etc.) as potential sources of anthocyanin-based colorants. Until now, many studies have been conducted on the use of natural colorants obtained from different anthocyanin sources in food production [11-15]. However, there is no study in the literature on the use of CA juice concentrate as a natural colorant in food products. It has been reported in studies that anthocyanins play an important role with their positive effects on human health, as well as their use as natural colorants in foods by giving their bright red color [16-18]. Anthocyanins are limited in their use due to their purification difficulties and low stabilization [15,18]. The addition of natural colorants obtained from edible anthocyanin sources to foods is preferred today. In this study, crab apple, which was selected as a natural food colorant and new anthocyanin source, was added to apple marmalade and after storage at different temperatures and times, pH, titratable acidity (TA), soluble solid content (SSC), reflectance color, antioxidant activity (AA), total phenolic (TP), and total monomeric anthocyanin (TMA) changes as well as degradation kinetic parameters (k, $t_{1/2}$, E_a, Q_{10} and z) were calculated.

MATERIAL AND METHODS

Material

Crab apple was collected from trees planted for landscaping from Selcuk University Alaeddin Keykubat Campus (Konya, Turkey) in 2019.

Methods

Crab apple juice concentrate and apple marmalade production

Crab appleswere washed, after the stems were separated, they were pressed in a juicer to obtain juice. Solid particles were separated by centrifugation at 5000 rpm for 5 minutes and clear juice was concentrated up to the total soluble solid content (SSC) of 70 under vacuum at 50 ^{o}C. The concentrate was stored at -18 ^{o}C until to marmalade production. The apples were peeled and cut into small pieces by using a knife and homogenized by passing through a blender (Arçelik K-1260). After weighing the homogeneous fruit pulp mixture, sugar (1:1 ratio) was added and heat treated. When the temperature of the mixture reached at 80 ^{o}C, citric acid solution (50% w/v) was added to adjusted to pH 3.2. Heat treatment was applied until the ^{o}Bx was 66% and set to 101 ^{o}C final temperature. crab apple juice concentrate (2%) was added to marmalades. After cooling to the 93 ^{o}C, filled into the 200 mL of glass jars. And cooled to room temperature immediadetly.

Storage conditions and shelf life experiments

The jars filled with apple marmelade were stored in incubators (Es 120, Nuve, Turkey) at selected temperatures of 9, 22 and 35 ^{o}C for 6 months.

Determination of pH, TA, SSC and color parameters

The pH and TA values of the samples were measured potentiometrically with a pH meter (WTW Inolab model, Weilheim, Germany). The marmalade samples were dissolved in distilled water at appropriate ratios and filtered. pH values of the samples were recorded at 20 ^{o}C directly the diluted samples were titrated with an adjusted 0.01 N NaOH solution until the pH reached 8.1. TA was calculated in terms of malic acid and the results were given as g malic acid equivalent 100 g^{-1} [20]. SSCs of water diluted and filtered samples were measured using a refractometer (Atago HSR-500, Japan) [20]. *L**, *a**, *b**, *h* and *C** values of the samples were measured with Konika Minolta CM-5 model colorimeter (Konika-Minolta, Osaka, Japan) [21].

Extraction procedure for TPC, TMA contents and antioxidant activity analyses

The extraction procedures applied in Coklar and coauthors [22] were modified. A 5 g of marmalade was weighed and extracted with 10 mL of methanol and by using an ultrasonic water bath (Transsonic TI-H-10, Singen, Germany) at 35 kHz frequency and 50% power for 30 minutes at 30 ºC for TPC and antioxidant activity analyses. For extraction of anthocyanins, 2 g of marmalade was extracted with 10 mL of acidified methanol (0.01%) by using the ultrasonic water bath as described above. Both phenolic and anthocyanin extracts were centrifugated (NF 800 R, Nuve, Turkey) for 10 minutes at 7000 rpm. Then, while supernatant of non-acidified methanol was taken for the analysis of TPCs and antioxidant activity analyses, supernatant of acidified methanol extract (5 mL) was evaporated under vacuum in a rotary evaporator to remove the methanol. The anthocyanin extract was re-suspended in a 0.5 mL methanol and used in TMA analysis.

TPC analysis

To determine the TPCs, 2.5 mL of 0.2 N folin-ciocalteu (Merck 109001, USA) reagent and 2 mL of sodium carbonate (Merck 106392, USA) (75 g L^{-1}) were put into 0.5 mL of extract and incubated for 2 h in room temperature. After 2 h, absorbance was measured at 765 nm in a spectrophotometer. Results were given as mg gallic acid equivalent kg^{-1} [23].

ABTS and DPPH antioxidant activity analyses

2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods were used to evaluate the antioxidant activities of the samples. To determine the ABTS antioxidant activity of the samples, 990 µL ABTS (Merck 11557, USA) solution generated with potassium persulfate (Merck 216224, USA) solution (2.45 mM) and ABTS solution (7 mM) was added to 10 µL of the extract. After incubation for 6 minutes, the decrease in the absorbance at 734 nm was recorded. Antioxidant values of extracts were given in mmol trolox equivalent TE kg^{-1}. To determine the DPPH antioxidant activity, 3.9 mL of DPPH (Aldrich D9132, USA) solution ($6x10^{-5}$ M) was added to 0.1 mL of diluted sample. After 30 minutes, the absorbance values at 515 nm wavelength were measured and the antioxidant activity values of the samples were calculated according to the calibration graph drawn with Trolox. Results were given as mmol Trolox equivalent kg^{-1} [24].

TMA content analysis

The TMA contents in the concentrate and marmalade samples were determined using the pH differential method described [25]. A 1 mL of extract was transferred into two different tubes. The first tube was diluted with 4 mL of pH 1.0 buffer (potassium chloride, 0.025 M) and the second was diluted with pH 4.5 buffer (sodium acetate, 0.4 M), separately. After 30 min, the wavelengths at 510 and 700 nm were measured using a spectrophotometer (U-1800, Hitachi, Japan), and the absorbance difference was calculated according to Equation 1. The TMA contents of the samples were calculated according to Equation 2 and the results were expressed in mg cyanidin-3-galactoside equivalent kg^{-1}

$$A = (A_{510nm}-A_{700nm})_{pH1.0}-(A_{510nm}-A_{700nm})_{pH4.5} \quad (1)$$

$$\text{Monomeric anthocyanin content (mg/L)}=(A \times MW \times DF \times 1000)/(\varepsilon \times l) \quad (2)$$

Where; MW: Molecular weight for cyanidin-3-galactoside, ε: Molar extinction coefficient for cyanidin-3-galactoside (L x mol^{-1} x cm^{-1}), l: Path lenght in cm, DF: Dilution factor

Calculation of kinetic parameters of anthocyanin degradation

Degradation of crab apple anthocyanins in the apple marmalade followed a first-order reaction law. First-order reaction law is defined by Equation 3 and taking integration of the Equation 3 gives the Equation 4 for integrated first-order reaction rate law.

$$-\frac{d[C]}{dt} = k[C]^n \quad (3)$$

$$C = C_o e^{-kt} \quad (4)$$

Where; k: Rate constant, C_o: Initial concentration, C: Concentration after t, t: Time

To determine the effect of temperature on anthocyanin degradation throughout the storage, activation energy (Ea) values were calculated via Equation 5.

$$k = k_o e^{-\frac{E_a}{RT}} \quad (5)$$

where; k: Rate contant, k_o: Pre-exponential factor, R: Ideal gas constant, J/mol ºK, Ea: Activation energy, J/mol ºK, T: Temperature

Temperature Coefficient (Q_{10}) values for anthocyanin degradation was calculated according to the Equation 6.

$$Q_{10} = \left(\frac{k_2}{k_1}\right)^{\frac{10}{(T_2-T_1)}} \quad (6)$$

The thermal resistance coefficient (*z value*) was calculated by Equation 7.

$$z = \frac{\ln 10\ R\ (T_2 T_1)}{E_a} \quad (7)$$

Half-life ($t_{1/2}$) was calculated by –ln(0.5)/k.

Statistical analysis

For the apple marmalade samples to which crab apple juice concentrate was added, during the 6-month storage period, 3 different storage temperatures; the effects on pH, water soluble dry matter, TA, reflectance color analysis, TPC and AA were carried out in two replications (6×3×2) factorial design. The data obtained as a result of the analyzes were evaluated using the variance analysis technique. According to the results of analysis of variance, Tukey's test was used to investigate which levels of the factors were important.

RESULTS

Some properties of crab apple juice concentrate

Some properties of the concentrate obtained from crab apple fruit used in the coloring of apple marmalade samples are given in Table 1. The pH value of crab apple juice concentrate (70 °Bx) used in our study was calculated as 2.90 and TMA amount as 442.47 mg kg^{-1}. *L*, a*, b*, C** and *h* color values were found as 14.89, 46.43, 24.86, 52.67 and 28.16, respectively. It has been stated in studies that wild apple varieties should be evaluated in food and beverages for reasons such as containing bioactive components (vitamin C, dietary fiber, antioxidants etc.) and attractive color [22,26]. There is no literature information on the use of crab apple selected for the study as an anthocyanin source in food products. However, some properties of concentrates obtained from anthocyanin sources obtained from different fruit-vegetable sources showed parallel findings with this study. Coklar and coauthors [22] found pH value of *Malus floribunda coccinella* to be 2.89, TA value to 2.21, SSC to 8.32, color parameters (*L*, a*, b**, C* and *h*) to be 22.54, 17.46, 3.65, 16.64, 12.29 in the fruit peel and 27.23, 29.42, 6.56, 30.2, 12.47 in the meaty part. TMA was found to be 3.68, 2.66 and 2.15 mg g^{-1} dry weight in the peel, whole fruit and fleshy part of the fruit, and the TPC was 56.85, 45.91 and 36.12 mg g^{-1} dry weight, respectively. The anthocyanin contents of crab apple juice concentrate used in this study was higher than strawberry [27], cherry [28], blackberry [29] and lower than black carrot [30].

Table 1. Some properties of crab apple juice concentrate

Parameters	Values
pH	2.90 ± 0.01
TA (g 100 g^{-1})	9.09 ± 0.00
SSC (%)	70.00 ± 0.00
TMA (mg kg^{-1})	442.47 ± 9.19
TPC (mg GAE g^{-1})	29.66 ± 0.18
*L**	14.89 ± 0.68
*a**	46.43 ± 1.05
*b**	24.86 ± 1.13
*C**	52.67 ± 1.46
h	28.16 ± 0.55

Effect of concentrate addition to marmalade

The analysis results of the apple marmalade sample colored with crab apple juice concentrate and sample produced without the addition of concentrate are shown in Table 2. Addition of crab apple juice concentrate in apple marmalade samples caused a decrease in pH and SSC, and an increase in TA. Color values showed a decrease in *L** and h values, and an increase in *a** and C* values of the concentrated added sample. In the study, the addition of crab apple juice concentrate provided new and attractive coloring in

apple marmalade samples. The addition of concentrate caused a numerical increase in both AA and TPC in apple marmalade.

Table 2. Some properties of colored and noncolored marmalade samples

Parameters	Non-colored Marmalade	Colored Marmalade
pH	3.47 ± 0.02	3.35 ± 0.01
TA (g 100 g^{-1})	0.31 ± 0.01	0.49 ± 0.02
SSC (%)	65.69 ± 2.40	67.52 ± 0.21
*L**	24.76 ±0.09	17.28 ± 1.02
*a**	2.70 ± 0.98	16.61 ± 1.57
*b**	15.42 ± 0.51	9.24 ± 1.86
C*	15.81 ± 0.79	18.82 ± 2.22
h	80.01 ± 3.47	28.10 ± 2.79
DPPH	1.16 ± 0.01	2.93 ± 0.07
ABTS	2.26 ± 0.24	5.72 ± 0.26
TPC (mg GAE g^{-1})	358.41 ± 46.46	863.28 ± 57.66
TMA (mg kg^{-1})	-	15.08 ± 0.92

Changes in pH, TA, SSC and color parameters of marmalade during storage

The pH values of the apple marmalades samples were found between 3.31-3.39. The lowest pH values were found in the samples stored at 22 ^{o}C for 3 and 6 months, and the highest values were found in samples stored at 22 and 35 ^{o}C for 1 and 2 months (Table 3.). While the effects of storage temperature and storage time on pH values of the samples were found to be statistically significant ($p<0.05$) their interaction was insignificant ($p>0.05$). In the literature, there are studies investigating the pH changes in marmalades produced using different fruits are stored both at different temperatures and times [31-34]. The pH value of traditional marmalades prepared with *Malus sylvestris* Miller (sour apple) wild fruit was measured as 2.66 [35,36] in their study, the initial pH value of strawberry marmalade samples was measured as 3.2. The pH values of the samples stored at room temperature at the 3rd and 6th months were 3.5 and 3.9. Acidity plays an important role in the formation of flavor balance and quality perception of foods [37] and also represents the stability and shelf life of food products. The acidity value is due to the organic acids naturally found in the fruits and the acid added while preparing the marmalade [38]. Acidity regulators provide the desired gelling by increasing the natural fruit flavor and the natural acid content from the fruit in marmalade production. In our study, TA values are between 0.40-0.50 in apple marmalades (Table 3). TA decreased compared to the initial values due to the increase in storage temperature and time in marmalades. Loss of organic acid in samples due to the increase of time and temperature factors may also cause a decrease in TA. According to the results, the effect of storage temperature and time on TA levels was found to be statistically significant ($p<0.05$). Besides, the effect of temperature-time interactions was statistically insignificant ($p>0.05$). In the previous studies, the TA of pumpkin [39] and [40] rosehip marmalade was measured as 0.24% and 0.9%, respectively. The TA of traditional marmalades prepared with *Malus sylvestris* Miller (sour apple) wild fruit was determined as 1.81% [36]. The TA of blueberry marmalade samples was initially around 0.65 on average, and 0.69, 0.66 and 0.78 at different storage times at room temperature (2, 4 and 6 months) [41]. Kaplan and Zühal [42] measured the initial TA of jujube marmalades as 0.08%. TA was recorded as 0.09, 0.09 and 0.11 at 4 and 20 ^{o}C 1, 2 and 3 storage times, respectively. When the studies on various fruit marmalades were examined, it was seen that the researchers found different TA and pH results. These differences are thought to be caused by factors such as production technique (traditional method/industrial type), the ratio of citric acid added, cooking degree and time, applied storage conditions and duration. The amount of SSC of marmalades is due to both the fruit used and the components added during production (pectin, acidity regulator, sucrose, etc.). SSC of apple marmalades produced in this study were determined in the range of 64.27-78.05%. While the initial SSC of apple marmalade with concentrated addition were 67.52%, these values were measured as 68.62%, 69.92%, and 67.35% after 6 months of storage at 9, 22 and 35 ^{o}C, respectively. Therefore, SSC values increased in apple marmalade depending on the increase in storage temperature and time (Table 3). According to the variance analysis, while storage time was statistically effective on SSC ($p<0.05$), storage temperature and storage temperature-time interaction had no effect on SSC ($p>0.05$). ^{o}Bx values of marmalades produced using four cultivars of raspberry fruits (*Rubus idaeus L.*) (Aksu Kırmızısı, Rubin, Hollanda Boduru and Heritage) and three cultivars of blackberry fruits (*Rubus*

fruticosus L.) (Bursa 1, Bursa 2 and Chester) was found in the range of 67.42 g/100-70.08 g^{-1} 100 g [43]. Güzel and coauthors [41] showed that the initial ºBx of blueberry marmalade was 60.00 and these values were 59.33, 61.36 and 59.76 at different storage times (2, 4 and 6 months), respectively. In another study, the ºBx values of persimmon marmalades were determined to be in the range of 63.1-66.5%. As storage times decreased in the marmalade samples, a decrease was observed in the SSC ratio [44]. The variety of bioactive components and color in the final product vary depending on the natural pigments in the raw material, the processing and the storage conditions [45, 46]. The color parameters measured as a result of storing the apple marmalades colored with crab apple juice concentrate at different temperatures and times are given in Table 4. While the *L** value was 24.76 in the apple marmalade samples produced without the addition of concentrate, it was measured as 17.28 in the samples with the concentrate addition. Although there were fluctuations in *L** values of apple marmalades stored at 9, 22 and 35 ºC for 6 months, it was determined that *L** values decreased as temperature and time increased. *L** values were determined in the range of 14.21-22.66 in marmalades during storage. The decrease in *L** values used as browning index in foods shows that color of samples darkened. *a** values in apple marmalades tend to decrease as expected with increasing storage temperature and time. While the *a** value of apple marmalades at the beginning of storage was 16.61, it decreased to 15.48, 13.00 and 11.84 after 6 months of storage at 9, 22 and 35 °C, respectively. The decrease in color parameters, especially *a** values, is the degradation of anthocyanin pigments depending on the increase in storage temperature and time. In the studies conducted with samples containing anthocyanin, it is stated that *a** values decrease depending on the prolongation of the storage period and the increase in temperature. In pomegranate jams [47], strawberry marmalades colored with black carrot anthocyanins [35,48] and Turkish delights [49] *a** value decreased depending on the increase in storage temperature and time. Concentrate added to marmalades reduced the *b** value, originally measured as 15.42, to 9.24. Although there were increases and decreases in the *b** values of the samples during the storage periods, this parameter increased as the storage temperature increased. The highest *b** value was found in samples stored at 35 ºC for 2 months (17.54), and the lowest *b** value was found in samples stored at 9 ºC for 3 months (8.23). It was determined that *b** values generally increased as the storage temperature and time increased in strawberry marmalades colored with black carrot anthocyanins [48] and Turkish delights [49]. The *C** value of apple marmalade with concentrated added was initially 18.82. This value was in the range of 16.41-22.98 in samples stored at 9, 22 and 35 ºC. This value decreased in general with increasing storage temperature and time. Martinsen and coauthors [46] reported that storing strawberry and raspberry jams at 23 ºC caused a paler color and decreased *C** value compared to storage at 4 ºC. The initial measured *h* value of apple marmalades produced without the addition of concentrate was 80.01, and 28.10 in those produced with the addition of concentrate. This decrease generally increased with the increase in storage temperature and time. The highest *h* value (52.79), which expresses the color tone, was measured in samples stored at 35 ºC for 6 months. More color loss with increasing storage temperature is attributed to the degradation of anthocyanin pigments and formation of brown compounds such as Maillard reaction products [35,45,50]. The effects of storage time, which are sources of variance, on *L**, *a**, *b**, *C** and h values were found to be statistically significant ($p<0.05$). The effect of interaction on *L**, *b**, *C** and *h* values was statistically insignificant ($p>0.05$). While the effect of storage temperature on *a**, *b** and *h* values was statistically significant ($p<0.05$), its effect on *L** and *C** values was insignificant ($p>0.05$).

Table 3. pH, TA and SSC values of apple marmalades stored at different temperatures and durations

Storage Temperature (°C)	Storage Period (day)	pH	TA (g 100g^{-1})	SSC (%)
9	30	3.36 ± 0.00	0.48 ± 0.02	67.93 ± 0.40
	60	3.36 ± 0.01	0.47 ± 0.00	77.59 ± 5.70
	90	3.33 ± 0.04	0.46 ± 0.02	72.77 ± 3.28
	120	3.35 ± 0.01	0.49 ± 0,02	66.88 ± 2.04
	150	3.33 ± 0.01	0.48 ± 0.01	64.27 ± 0.22
	180	3.33 ± 0.00	0.50 ± 0.00	68.62 ± 2.82
22	30	3.39 ±0.01	0.48 ± 0.02	66.86 ± 0.17
	60	3.39 ± 0.01	0.45 ± 0.01	76.68 ± 2.99
	90	3.31 ± 0.01	0.44 ± 0.03	74.23 ± 6.05
	120	3.35 ± 0.04	0.48± 0.01	65.72 ± 1.74
	150	3.35 ± 0.03	0.48 ± 0.02	66.44 ± 0.67
	180	3.31 ± 0.01	0.47 ± 0.03	69.92 ± 0.12
35	30	3.39 ± 0.01	0.47 ± 0.01	68.85 ± 1.89
	60	3.39 ± 0.01	0.45 ± 0.01	76.62 ± 2.48
	90	3.37 ± 0.00	0.40 ± 0.01	78.05 ± 4.57
	120	3.36 ± 0.01	0.45 ± 0.02	65.74 ± 1.63
	150	3.37 ± 0.02	0.45 ± 0.01	66.47 ± 1.82
	180	3.37 ± 0.03	0.45 ± 0.01	67.35 ± 0.64

Table 4. *L**, *a**, *b**, C* and *h* values of marmalade samples stored at different temperatures and times

Storage Temperature (°C)	Storage Period (day)	*L**	*a**	*b**	C*	*h*
9	30	18.80 ± 2.9	17.45 ±2.5	10.64 ± 2.9	20.45 ±3.73	31.07 ± 3.32
	60	21.01 ± 2.9	17.87 ±2.1	11.41 ± 2.08	21.21 ±2.94	32.43 ± 1.61
	90	15.68 ± 1.0	14.39 ±0.68	8.23 ± 1.09	16.41 ±1.37	30.04 ± 1.61
	120	18.01 ± 1.9	14.62 ±0.42	10.12 ± 1.54	17.80 ±1.23	34.58 ± 3.30
	150	16.76 ± 0.1	14.10 ±0.49	9.65 ± 0.25	17.01 ±0.65	34.57 ± 0.47
	180	18.80 ± 0.4	15.48 ±1.3	10.47 ± 0.94	18.82 ±1.85	33.83 ± 0.30
22	30	21.46 ± 0.2	18.45 ±0.4	13.10 ± 0.47	22.63 ±0.59	35.37 ± 0.41
	60	21.83 ± 3.3	16.35 ±1.4	13.33 ± 1.68	21.10 ±2.16	39.13 ± 1.13
	90	18.22± 1.5	14.29 ± 1.12	12.25 ± 1.77	18.83 ±2.00	40.52 ± 1.89
	120	17.79 ± 0.0	13.25 ± 0.92	12.32 ± 0.33	18.09 ±0.89	42.93 ± 1.19
	150	16.28 ± 0.5	12.21 ± 0.98	11.60 ± 1.53	16.84 ±1.76	43.44 ± 1.52
	180	19.20 ± 0.1	13.00 ± 0.20	13.15 ± 1.35	18.50 ±1.10	45.24 ± 2.51
35	30	22.20 ± 0.5	15.94 ± 0.82	16.55 ± 1.38	22.98 ±1.56	46.05 ± 0.92
	60	22.66 ± 1.0	13.62 ± 0.08	17.54 ± 1.18	22.21 ±0.98	52.14 ± 1.70
	90	14.29 ± 0.5	10.58 ± 0.87	13.04 ± 1.17	16.79 ±1.46	50.88 ± 0.11
	120	16.54 ± 0.7	11.36 ± 0.52	14.70 ± 1.26	18.58 ±1.32	52.24 ± 1.08
	150	14.21 ± 0.2	10.43 ± 0.81	13.48 ± 0.74	17.04 ±1.07	52.29 ± 0.62
	180	16.95 ± 1.1	11.84 ± 0.4	15.64 ± 1.94	19.63 ±1.82	52.79 ± 2.40

Changes in TPC and AA during storage

The high phenolic content of the samples at the beginning of storage may be associated with high anthocyanin content. Although there were some fluctuations in the TP of apple marmalade during storage, the increase in temperature and time decreased the phenolic composition of the samples (Table 5.). The amount of phenolic substance in apple marmalades varied between 572.48-858.77 mg GAE kg^{-1}. It is thought that phenolic substances may be decomposed during storage. The effect of storage temperature and time on TPC was found to be statistically significant ($p<0.05$). The interaction was found to be insignificant ($p>0.05$)

in apple marmalades. Yildiz and Alpaslan [40] measured the TPC of rosehip marmalades as 912.4 GAE mg $100g^{-1}$. Başkaya Sezer and coauthors [51] measured the TPC values of marmalades of two different plum varieties (Yonuz and Jackal plum) as 45.67 and 47.75 mg GAE $100g^{-1}$, respectively. There are many studies in the literature that the amount of phenolic substances decreases in parallel with the increase in temperature during storage. From these studies, the initial TPC (µg GAE g^{-1}) of the blueberry marmalade samples was 391.45. At the end of storage periods of 2, 4 and 6 months at room temperature, these values were calculated as 332.15, 313.7 and 237.65 [41]. The TPC of strawberry fruit decreased to 8503.13 mg GAE kg^{-1}, and after jam production it decreased to 578.26 mg GAE/kg. This value was reported as 507.61, 487.68, 476.81, 467.75 and 455.07 mg GAE kg^{-1}, respectively, in samples stored at 25 ^{o}C for 5 months and taken every month [32]. The initial TPC of the strawberry marmalade samples was specified as 156.8 mg 100 g^{-1}. The TP values of the samples stored at room temperature were measured as 138.7 and 114.5 mg 100 g^{-1} at the 3rd and 6th months. The initial TP value of the samples enriched with black carrot puree (30%) was 212.5 mg 100 g^{-1}, 204.8 mg 100 g^{-1} at room temperature for 3 months, and 198.4 mg 100 g^{-1} after 6 months of storage [35]. Fruits are recognized as an excellent source of bioactive phenolic compounds [32]. During fruit processing, cell structures are disrupted and fruits become more prone to non-enzymatic oxidation, which may be one of the main causes of loss in phenolic compounds [32,50]. Mazur and coauthors [52] suggested that the loss or formation of different bioactive compounds in foods may be affected by both different processing methods and interactions between phytochemicals and different food ingredients during storage. Therefore, it can be concluded that the processing and storage conditions have a negative effect on the TP, and the percentage of loss varies according to the type of fruit, fruit variety and jam composition [53]. The addition of crab apple juice concentrate increased the ABTS values of apple marmalades from 2.26 to 5.72 mmol TE kg^{-1}. The increase in storage temperature and time in marmalade samples significantly decreased the AA values determined by ABTS method. In the apple marmalade samples, the highest value was determined in the samples stored at 5.40 to 9 ^{o}C for 2 month, and the lowest value was detected in the samples stored at 2.04 to 35 ^{o}C for 6 months (Table 5.). The DPPH values of marmalades colored with crab apple juice concentrate gave higher values than the samples without added concentrate. The initial DPPH value of apple marmalades with concentrated addition was determined as 2.93 mmol TE kg^{-1}. The increase in storage temperature and time in apple marmalades significantly decreased the antioxidant capacity results determined by the DPPH method. The highest values in marmalades were found in the samples stored at 9 ^{o}C for 1 month, and the lowest values were found in the samples stored at 35 ^{o}C for 6 months. According to the results of the DPPH method, the AA levels detected in apple marmalades are in the range of 1.76-2.90 mmol TE kg^{-1} (Table 5.). The effects of storage temperature and storage time on DPPH and ABTS values were found to be statistically significant ($p<0.05$). On the other hand, the temperature-time interactions were found to be insignificant on DPPH ($p>0.05$) and significant on ABTS ($p<0.05$) values. In a previous study [54], DPPH radical scavenging activity (% inhibition) of rosehip marmalade was determined as 7.23. The total antioxidant capacity of black carrot marmalades was recorded as 1200 mg TE 100 g^{-1} dry weight. After 4, 8, 12, 16 and 20 weeks of storage at 4 ^{o}C, these values were found to be 1203, 1218, 1127, 1115 and 1035, respectively, and at 25 ^{o}C these values were found to be 1142, 1140, 1124, 1120 and 801 mg TE 100 g^{-1} dry weight [55]. Amakura and coauthors [56] reported that the DPPH radical scavenging activity value of the strawberry jam samples they produced was 2.16 mg mL^{-1}. While the initial DPPH free radical scavenging activity value of jams produced from goji berries was 60.98%, it was 60.31% after 5 days of storage at refrigerator temperature and 59.56% at the end of 10 days of storage [34]. Rababah and coauthors [32] reported that the DPPH (% inhibition) value of strawberry fruit decreased to 54.88 and 42.50 after jam production. These values changed as 33.08, 33.53, 33.60, 33.05 and 28.49, respectively, in the samples stored at 25 ^{o}C for 5 months and taken every month. As with the research findings on the total amount of phenolic substances, the results of AA also showed differences between studies. It is thought that the reason for the different results found may be due to the raw materials used in the research, the extraction applied, the analysis methods and the unit differences in the results given. However, the decrease in AA with the increase in storage temperature and time is consistent with the results we obtained in our study.

Table 5. TPC, ABTS and DPPH analysis of marmalade samples stored at different

Storage Temperature (°C)	Storage Period (day)	TPC (mg GAE kg^{-1})	ABTS (mmol TE kg^{-1})	DPPH (mmol TE kg^{-1})
9	30	832.78 ± 29.92	5.37 ± 0.18^{a}	2.90 ± 0.29
	60	858.77 ± 18.75	5.40 ± 0.10^{a}	2.86 ± 0.05
	90	839.75 ± 98.57	4.91 ± 0.78^{ab}	2.82 ± 0.08
	120	853.29 ± 29.38	5.11 ± 0.16^{a}	2.70 ± 0.02
	150	750.58 ± 3.41	4.46 ± 0.34^{abc}	2.54 ± 0.21
	180	706.46 ± 27.88	4.29 ± 0.37^{abc}	2.54 ± 0.04
22	30	823.90 ± 54.58	5.04 ± 0.05^{a}	2.83 ± 0.11
	60	803.61 ± 7.80	4.63 ± 0.13^{ab}	2.73 ± 0.11
	90	761.80 ± 17.03	4.60 ± 0.11^{ab}	2.52 ± 0.06
	120	794.55 ± 106.09	4.43 ± 0.04^{abc}	2.42 ± 0.02
	150	600.08 ± 39.63	3.32 ± 0.11^{c-f}	2.32 ± 0.01
	180	603.87 ± 24.59	3.42 ± 0.12^{cde}	2.18 ± 0.08
35	30	838.18 ± 36.46	4.48 ± 0.64^{abc}	2.42 ± 0.18
	60	654.24 ± 55.14	3.77 ± 0.27^{bcd}	2.15 ± 0.04
	90	666.15 ± 9.28	2.74 ± 0.21^{d-g}	2.04 ± 0.01
	120	662.05 ± 63.58	2.45 ± 0.15^{efg}	1.97 ± 0.13
	150	598.72 ± 54.84	2.20 ± 0.08^{fg}	1.85 ± 0.04
	180	572.48 ± 11.79	2.04 ± 0.01^{g}	1.76 ± 0.04

Changes in TMA amount during storage

The anthocyanin content of the colored apple marmalade was found to be 15.08 mg kg^{-1}. As seen in Figure 1, the total amount of monomeric anthocyanin in the colored apple marmalades decreased as the storage temperature and time increased. Losses in total monometric anthocyanin content of apple marmalades stored at 9, 22 and 35 °C for 6 months were determined as 31.29%, 51.92% and 87.33%, respectively. According to Mazur and coauthors [52] reported the highest TMA content as 28.3 mg 100 g^{-1} in jams produced from red raspberries of different genotypes. This value decreased to 16.6 and 11.9 mg 100 g^{-1} in samples stored at 20 ºC for 3 and 6 months, respectively. The initial total anthocyanin content value of strawberry marmalades enriched with 30% black carrot puree was calculated as 58.9 mg 100 g^{-1}, 56.4 mg 100 g^{-1} for samples stored at room temperature for 3 months, and 54.6 mg 100 g^{-1} for samples stored for 6 months [35]. It is understood that the losses in the amount of anthocyanins obtained in the studies vary depending on the reasons such as the raw material used, the source, amount of anthocyanins, storage temperature and time.

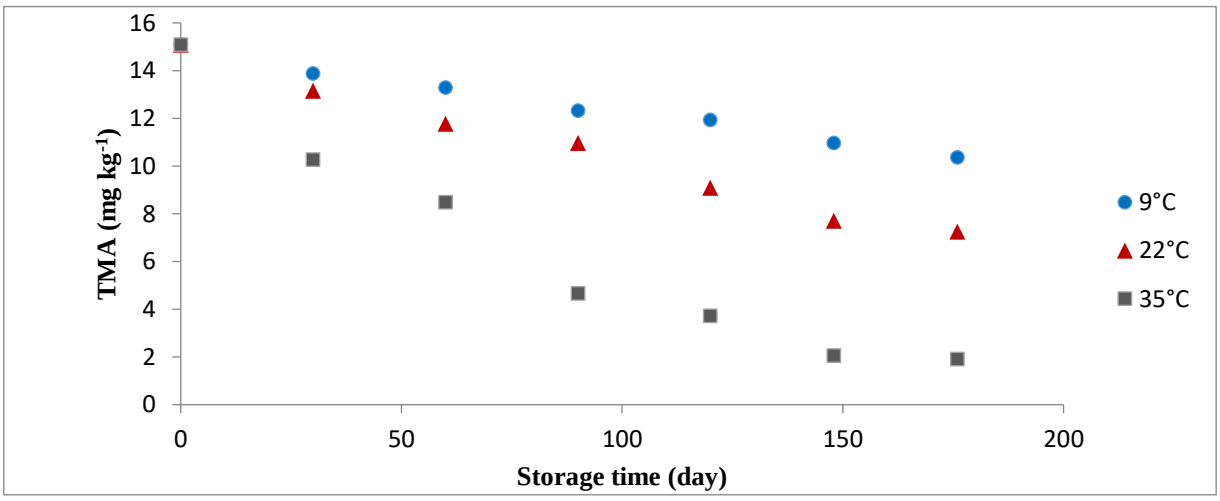

Figure 1. Changes in anthocyanin amounts as a result of storing apple marmalades colored with crab apple at different temperatures

Degradation kinetics

The samples colored with crab apple juice concentrate were stored for 6 months at different temperatures and the reaction rate constants (k) were determined by using the values obtained as a result of the analysis of the TMA content of the samples. Based on the reaction rate constants, the half-lives ($t_{1/2}$), activation energy (Ea), Q_{10} and z values of the samples were calculated. Degradation of crab apple anthocyanins followed the first-order reaction. It has been stated by many researchers that the degradation of anthocyanins conforms to the first-order reaction kinetics during both heating and storage [1,15,29,30,33,48,50,57-61]. The kinetic parameters of the degradation of anthocyanins during storage of marmalades with added crab apple juice concentrate at 9, 22 and 35 ^oC are shown in Table 6. As the storage temperature of the samples increased, the reaction rate constants increased. The rate constants were determined as $2.1x10^{-3}$ day^{-1}, $4.3x10^{-3}$ day^{-1} and $12.4x10^{-3}$ day^{-1} in apple marmalades stored at 9, 22 and 35 °C, respectively. Studies have shown that as the storage temperature increases, the k value increases [29,30,62].Looking at the half-life of the samples in Table 6, it is seen that it decreases with the increase in storage temperature. The half-life of anthocyanins in apple marmalades stored at 9, 22 and 35 °C were 330.07, 161.20 and 55.90 days, respectively. This indicates that the storage stability of crab apple anthocyanins decreases as the storage temperature increases. Other authors also reported that $t_{1/2}$ values decrease with increasing temperature [29,48,59,63,64]. The higher the activation energy, the more sensitive the reaction rate to temperature change. The activation energy in apple marmalade was determined as 49.20 kJ mol^{-1}. Table 6 shows how many times the reaction rates of the samples increase with temperature increase between 9-22 ^oC, 9-35 ^oC and 22-35 ^oC for apple marmalades. Considering the average value in all temperature ranges given, it was determined as 1.99. There is an inverse ratio between the z value and the E_a value. The higher the z value, the less the reaction is affected by temperature changes. According to Table 6. the z value was calculated as 33.88 ^oC. In all samples stored at high temperatures, a very rapid loss of anthocyanins occurred in a short time, and the stability of anthocyanins increased as the storage temperature decreased. In the researches, pomegranate jam [47], blueberry jam [59], sour cherry jam [60], raspberry jam [46], blackberry jam [33], black carrot jam and marmalade [55] reported that anthocyanins were more preserved with low temperature storage. No study has been found in the literature on the storage stability of crab apple anthocyanins in food products. In the study conducted with crab apple juice, the degradation of anthocyanins occurred in accordance with the first-order reaction kinetics at the end of the heat treatment applied at 70, 80 and 90 ^oC. The rate constants of crab apple anthocyanins at 70, 80 and 90 ^oC were 1.70, 3.30 and 6.90 x 10^{-3} min^{-1}, their half-lives were 6.80, 3.50 and 1.68 hours, respectively, and the activation energy was 72.45 kJ mol^{-1} [65]. Although there are many studies on the degradation kinetics of anthocyanins in fruit juices and concentrates [62,66-68], studies on the degradation of anthocyanins in jams and marmalades are very limited. The storage stability of anthocyanins was investigated in blackberry juice (8.90 ^oBx) and blackberry juice concentrate (65.0 ^oBx) at 5, 25 and 37 ^oC for 2 months. While the k values of blackberry juices were 2.0, 21.6 and 59.1 $x10^{-3}$ min^{-1}, respectively, these values were determined as 5.2, 36.4 and 89.9 $x10^{-3}$ min^{-1} in blackberry juice concentrates. $t_{1/2}$ values were calculated as 330.1, 32.1 and 11.7 days for fruit juices and 138.6, 19.7 and 9.4 days for concentrates, and activation energies were calculated as 75.5 kJ mol^{-1} for fruit juices and 65.06 kJ mol^{-1} for concentrates [29]. While k values were 1.15, 5.07 and $23.03x10^{-3}$ $days^{-1}$, $t_{1/2}$ values were 603, 137 and 29 days, respectively, in black carrot juice concentrate (68 ^oBx) samples stored at 5, 20 and 30 ^oC; the E_a value was stated as 84 kJ/mol [30]. The k value of strawberry nectar, which is prepared by adding sucrose at a concentration of 20% and stored at 20 °C for 42 days, is 26.5 $x10^{-2}$ $days^{-1}$ and its half-life is 26 days [58]. In apple juice samples colored with purple sweet potato anthocyanins, $t_{1/2}$ values at 80, 90 and 100 ^oC were determined as 32.4, 17.0 and 8.1 hours respectively, and E_a value was 75.68 kJ mol^{-1} [18]. After storage at 4, 20 and 37 °C, the k values of apple juices colored with black carrot juice concentrate (1.5 g 100 mL^{-1}) were 2.07, 8.75 and $59.9x10^{-3}$ $days^{-1}$, $t_{1/2}$ values were 47.8 weeks, 11.3 weeks and 1.7 weeks, and the E_a value was determined as 72.7 kJ mol^{-1} [69]. Examining the degradation of anthocyanins in strawberry jams stored at 4 and 15 °C, Patras and coauthors [50] stated the k values as 0.95×10^{-2} day^{-1} and 1.71×10^{-2} day^{-1}, respectively. The k values in blackberry jam samples stored at 10 and 25 ^oC for 180 days were calculated as 0.0024 and 0.0125 day^{-1}, respectively, the Q_{10} value was 3.0 and the E_a value was 19,490.33 kcal mol^{-1} [33]. When these values are compared with the results we have obtained, it shows that the storage stability is higher in apple marmalades with the addition of crab apple juice concentrate. In the study investigating the storage stability of strawberry jams enriched with black carrot juice concentrate, the rate constant was 2.53 x 10^{-3} $days^{-1}$ at 10 °C, $4.60x10^{-3}$ $days^{-1}$ at 22 °C and 14.50 $x10^{-3}$ $days^{-1}$ at 37 °C. $t_{1/2}$ values were 39.1, 21.5 and 6.8 weeks, respectively [48]. When we look at the kinetic parameters, according to the results we obtained in our study, the more stable anthocyanins can be explained by both the anthocyanins found in strawberries and the black carrots containing acylated anthocyanins. Different raw materials and components used in production,

anthocyanin composition of the samples, extraction conditions, and different storage temperatures and times are the main reasons for the different kinetic parameters of the degradation of anthocyanins in the studies.

Table 6. Kinetic parameters for the degradation of anthocyanins after storage of marmalade samples (*R^2 value)

Temperature (°C)	k *x* 10^{-3} (day^{-1})	$t_{1/2}$ (day)	Activation energy (kJ mol^{-1})	Z (°C)	Q_{10}			
					(9-22)	(9-35)	(22-35)	Average value
9	2.1 (0.992)*	330.07						
22	4.3 (0.987)	161,20	49.20 (0.982)	33.88	1.74	1.98	2.26	1.99
35	12.4 (0.980)	55,90						

CONCLUSION

The use of ingredients rich in natural bioactive compounds in food formulations will enable consumers to reach the natural products they prefer, thus contributing to a healthy diet. Studies on the use of red fruits, which are rich in antioxidant activity, phenolic compounds and anthocyanins, as natural alternatives to artificial colorants are increasing day by day. In this direction, it is important both to introduce new and alternative sources to the food industry and to investigate the stability of these sources in food products. According to the findings obtained in this study, it can be suggested that crab apple anthocyanins be used in food production as a new natural colorant source. At the same time, crab apple fruit will be able to functional properties to the food products to which it is added, thanks to its antioxidant activity. Increasing the use of natural anthocyanin sources such as crab apple instead of artificial colorants in food products will have positive effects on human health. When the analysis results obtained at the selected storage temperatures in our study were examined, the most appropriate results were determined in the samples stored at 9 °C.

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