Vol.66: e23220947, 2023
https://doi.org/10.1590/1678-4324-2023220947
ISSN 1678-4324 Online Edition

Article - Food/Feed Science and Technology

Evaluation the Storage Stability of Crab Apple (*Malus floribunda*) Anthocyanins, a Natural Antioxidant Colorant, in Turkish Delights

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Editor-in-Chief: Bill Jorge Costa
Associate Editor: Bill Jorge Costa

Received: 04-Dez-2022; Accepted: 30-May-2023

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HIGHLIGHTS

- Crab apples that could not be consumed were evaluated.
- Turkish delight is colored with crab apple anthocyanins instead of synthetic colorants.
- Crab apple phenolics have added functional properties to Rose-flavored Turkish delight.

Abstract: In this study, the usability of crab apple (*Malus floribunda*), which can be consumed as food but has limited economic use, as a natural colorant in Turkish delight was investigated. Crab apple concentrate was added to Turkish delight at a rate of 1.83% during production. Produced Turkish delight were stored at 22, 28 and 35 ^{o}C for 6 months and analyzed some physicochemical and biochemical parameters. With the increase in storage temperature and time, there were no significant changes in the titratable acidity compared to the initial values. As the storage temperature and time increased, the *L**, *a**, *C** values of all samples decreased, while the *b** and *h* values increased. The increase in temperature and time during the 6-month storage period caused a significant decrease in total phenolic, total monomeric anthocyanin and antioxidant activity values. Degradation of crab apple anthocyanins during storage occurred according to first-order reaction kinetics. The degradation rate constants of anthocyanin in Turkish delight stored at 22, 28 and 35 °C were determined as -4.2x10-3 days-1, -8.7x10-3 days-1 and -14.2x10-3 days-1, respectively. According to the results obtained, the addition of crab apple juice concentrate gave both desired color and functional properties to Turkish delight.

Keywords: Anthocyanin; crab apple; natural food colorant; Turkish delight; storage.

INTRODUCTION

Color is an important sensory feature in determining the quality of foods, and it also affects the taste and aroma expectations of consumers [1, 2]. The food industry uses colorants for purposes such as preserving the original appearance of foods, ensuring color uniformity of the product, and giving color to colorless foods. Food colorants are generally divided into three basic classes as synthetic, nature-identical, and natural [1, 3]. Synthetic colorants have advantages such as low cost, ease of application and stability. However, due to the health problems they may cause with their potential toxic effects and the increasing demand of consumers for food products formulated with natural ingredients, manufacturers are turning to natural colorants [4, 5]. Anthocyanins, one of the natural pigments, are water-soluble polyphenolic compounds naturally found in fruits, vegetables, flowers and cereal grains, offering orange, red, purple and blue colors [6]. Anthocyanins have antioxidant and bioactive properties that have been linked to certain health benefits such as anti-diabetic, anti-inflammatory and anti-cancer effects [7, 8].

Turkish delight (lokum) is a confectionery product that differs from the others with its characteristic soft and jelly-like consistency [9]. Coloring agents used in Turkish delight production are generally tartrazine (E102), sunset yellow (E110), orange yellow, ponceau 4R (E124), green S (E142), brilliant blue and carmoisine [10]. Studies on the use of natural colorant sources in Turkish delight production are very limited. Examples of anthocyanins used as colorants in foods are red grape, elderberry, blackcurrant, blackberry, raspberry, black chokeberry, red cabbage, black carrot, purple corn, red radish and purple sweet potato [11, 12]. In recent years, the search for new and alternative sources rich in anthocyanins and the possibilities of using anthocyanins as natural food colorants have increased [1, 5]. There is no study in the literature on the use of crab apple anthocyanins as a natural colorant in food products. In this study, crab apple juice was added to the Turkish delight samples during production. Changes in some physicochemical parameters were investigated during the storage at three different temperatures.

MATERIAL AND METHODS

Crab apple juice concentrate and Turkish delight production

Crab apple was collected from trees planted for landscaping from Selcuk University Alaeddin Keykubat Campus (Konya, Turkey) in September 2019. Crab apples were 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 of 70 under vacuum at 50 ^{o}C. The concentrate was stored at -18 ^{o}C until to Turkish delight samples production. Turkish delights were produced in the production facilities of Azim Confectionery Company in Konya, which produces on an industrial scale. In the first step of the production, water and sugar were mixed as much as water could dissolve and heating was applied. Meanwhile, citric acid was dissolved in some water in another vessel. In the remaining part of the water to be used, the starch was suspended. Afterwards, starch suspension and acid were added to the sugar solution and the mixture was cooked, while 550 g (1.83%) of crab apple juice concentrate with a brix value of 70 was added to 30 kg of Turkish delight mixture and colored. When the Turkish delight mass reached the desired consistency, it was removed from the fire, flavored with rose flavor, and poured onto starchy wooden trays and rested on the trays for 12-24 hours. A mixture of powdered sugar and starch was poured over the Turkish delight. It was packaged after being cut in the desired cubic shape. Polypropylene plastic packaging was used for packaging and the packaged products were placed in 1 kg cardboard boxes.

Storage conditions and shelf-life experiments

Produced Turkish delight samples were stored in incubators at selected temperatures of 22, 28 and 35 ^{o}C for 6 months. During the 6-month storage period, the specified analyzes were performed on the samples taken every month.

Determination of pH, titratable acidity, soluble solid content and color parameters

The pH and titratable acidity (TA) values of the samples were measured potentiometrically with a pH meter (WTW Inolab model, Weilheim, Germany). Twelve grams of Turkish delight samples were made up to 60 g with distilled water and dissolved. This solution was then filtered. pH values of the samples were recorded at 20 ^{o}C. 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 citric acid and the results were given as g citric acid equivalent/100g [13]. Soluble solid contents (SSC) of water diluted and filtered samples were measured using

a refractometer (Atago HSR-500, Japan) [14]. *L**, *a**, *b**, *h* and *C** values of the samples were measured with Konika Minolta CM-5 model colorimeter (Konika-Minolta, Osaka, Japan) [15].

Extraction procedure for total phenolic, total monomeric anthocyanin contents and antioxidant acitivity analyses

Five g of sample was extracted with 10 ml of methanol by using an ultrasonic water bath (Elma, Transsonic TI-H-10, Singen, Germany) at 35 kHz frequency and 50% power for 30 minutes at 30 ^{o}C for total phenolic (TP) compound and antioxidant activity analyses. For extraction of anthocyanins, 2 g of sample 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, Nüve, Turkey) for 10 minutes at 7000 rpm. Then, while supernatant of non-acidified methanol was taken for the analysis of TP compounds 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 resuspended in a 0.5 ml methanol and used in total monomeric anthocyanin (TMA) analysis.

Total phenolic compound analysis

To determine the TP compounds, 2.5 mL of 0.2 N folin-ciocalteu reagent and 2 mL of sodium carbonate (75 g/L) 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 [16].

ABTS and DPPH antioxidant activity analyses

ABTS and DPPH methods were used to evaluate the antioxidant activities of the samples. To determine the ABTS antioxidant activity of the samples, 990 µL ABTS$^{\bullet}$ solution generated with potassium persulfate 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 was recorded by a spectrophotometer at 734 nm. Antioxidant values of extracts were given in mmol Trolox equivalent (TE)/kg. To determine the 2,2-diphenyl-1- picrylhydrazyl (DPPH) antioxidant activity, 3.9 mL of DPPH solution (6 *x* 10^{-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 [17].

Total monomeric anthocyanin content analysis

The TMA contents in the concentrate and Turkish delight samples were determined using the pH differential method described [18]. One ml of the extract was transferred into two separate tubes. The first tube was diluted with 4 mL of pH 1.0 buffer (potassium chloride, 0.025 M) and the second tube was diluted with pH 4.5 buffer (sodium acetate, 0.4 M). After 30 min, absorbances at 510 and 700 nm of the samples were measured using a spectrophotometer (U-1800, Hitachi, Japan), and the absorbance differences were 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

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

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

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 length in cm, DF: Dilution factor

Calculation of kinetic parameters of anthocyanin degradation

Degradation of crab apple anthocyanins in Turkish delights was determined to be suitable for first-order kinetics, therefore first-order kinetics were applied to the data obtained during storage. First order kinetic is defined by Equation 3 and taking integration of Equation 3 gives the Equation 4. for integrated first-order reaction rate law.

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

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

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.

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

where; k: rate constant, k_o: pre-exponential factor, R: ideal gas constant, J/mol ^oK, Ea: activation energy, J/mol ^oK, T: Temperature (K)
Temperature Coefficient (Q_{10}) values for anthocyanin degradation was calculated according to the Equation 6.

$$Q_{10} = \left(\frac{k_2}{k_1}\right)^{\frac{10}{(T2-T1)}} \quad \text{Eq. (6)}$$

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

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

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

Statistical analysis

For the Turkish delight samples to which crab apple juice concentrate was added, during the 6-month storage period, 3 different storage temperatures; the effects on pH, soluble solid content, titratable acidity, reflectance color analysis, total phenolic substance and antioxidant activity 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

Changes in some physicochemical properties of Turkish delight samples during storage

Preliminary trials were carried out to match the color of the Turkish delight produced using synthetic colorants, and the desired color tone was adjusted with the addition of crab apple juice concentrate. Analysis results of Turkish delight samples produced by coloring with crab apple juice concentrate at the beginning of storage are given in Table 1. In the study, the addition of crab apple juice concentrate provided attractive coloring in Turkish delight samples. The pH value of Turkish delight sample was determined as 3.30 and ranged between 3.28-3.39 during the storage (Table 2). Although increases and decreases in pH values are detected during storage, the pH values are highest in samples stored at 35 ^oC for 6 months. According to variance analysis results, the effect of storage time on pH values of Turkish delight samples was statistically significant ($p<0.05$). It was determined that the effects of storage temperature and storage temperature-time interactions on pH values were insignificant ($p>0.05$). While the pH of the Turkish delight enriched with 2.5, 5 and 7.5% sour cherry juice concentrate (65 brix) is 3.53, 3.43 and 3.39, respectively, the pH value of the uncolored and unflavored Turkish delight produced as control is 4.03. The pH values of the Turkish delight enriched with 2.5, 5 and 7.5% black grape juice concentrate (65 brix) were determined as 3.71, 3.59 and 3.53, respectively [19]. The initial pH value of Turkish delight samples colored with black carrot concentrate was measured as 3.99. The pH values of the samples applied at different temperatures (12, 20 and 30 ^oC) and storage times (60, 90, 120 and 150 days) were determined. According to the results obtained; for samples stored at 12 ^oC, these values are 4.08, 4.15, 4.27 and 4.39, respectively; It is 4.02, 4.08, 4.12 and 4.14 for 20 ^oC and 4.12, 4.28, 4.35 and 4.58 for 30 ^oC [12]. It was determined that the initial pH value of the uncolored and unflavored Turkish delight samples produced was 4.11. The pH levels were measured as 4.05 after 10 weeks of storage at 18-25 ^oC and as 4.13 at the end of 20 weeks of storage. While the initial pH values of Turkish delight samples, in which 4.4% and 12.2% cranberry pulp were added, were 3.7 and 3.63, respectively, it was recorded as 3.57 and 3.62 at the end of 10 weeks, and as 5.22 and 3.55 at the end of 20 weeks [20]. In the study conducted by adding pomegranate seeds (17-19%) into the produced Sultan Turkish

delight, the initial pH of the samples was 4.83, while it was 4.51 after 2 months of storage, 4.05 after 4 months of storage and 3.39 after 6 months of storage [21]. When the results of the research with the values we have obtained are compared, it is seen that the pH values of the Turkish delight samples are different. It is thought that these differences are caused by factors such as production technique, raw materials used and different natural colorants, cooking degree and time, applied storage conditions and duration. The TA value in Turkish delight samples, which was initially measured as 0.21 g/100 g, did not show a significant change during storage at different temperatures. TA values were found to be 0.21-0.23 g/100g during storage (Table 2). The effect of storage time on TA values in Turkish delight samples was found to be statistically significant ($p<0.05$). It was determined that the effects of storage temperature and temperature-time interactions on TA were insignificant ($p>0.05$).

Table 1. Some quality characteristics of colored Turkish delight samples

Properties	**Values**
pH	3.30±0.00
TA (g/100 g)	0.21±0.00
SSC (%)	74.67±0.53
*L**	30.56±0.13
*a**	2.96±0.02
*b**	-0.79±0.03
*C**	3.09±0.00
h	344.04±0.45
DPPH (mmol TE/ kg)	3.43±0.04
ABTS (mmol TE/ kg)	2.98±0.06
TPC (mg GAE/g)	542.54±13.14
TMA (mg/kg)	6.49±0.57

Batu and coauthors [22] reported the TA of uncolored and unflavored Turkish delight as 0.067% in a study they conducted. Batu, Arslan [19] reported that the TA value of uncolored and unflavored Turkish delight produced as control was 0.067%. The TA of Turkish delights enriched with 2.5, 5 and 7.5% cherry juice concentrate (65 brix) are 0.438%, 0.750% and 1.041%, respectively. In the Turkish delights produced by adding black grape juice concentrate (65 brix) at the same rates, TA levels were measured 0.170%, 0.280% and 0.347%, respectively. In the production made by adding pomegranate seeds in the range of 17-19% into Sultan Turkish delight, the initial total acidity value of the samples was calculated as 1.745%, after 2 months of storage 1.752%, after 4 months of storage 2.175 and at the end of 6 months of storage calculated as 2.182% [21]. In another study, the pH value of rose delight was 5.59 and the TA was determined as 0.11%. No significant differences were observed in terms of pH values and total acidity in the samples stored for 21 days in the dark at 20 ^{o}C [23]. When the literature studies on Turkish delight were examined, different titratable acidity values have reported depending on the raw material used. In our study, the fact that there was no significant change in the TA values with the increase in storage temperature and time in Turkish delight samples was in accordance with the results of previous studies. Soluble solid content (SSC) of Turkish delight samples stored at different temperatures and times were measured in the range of 74.89%-81.08%. While the initial SSC values of the Turkish delight samples to concentrated added were 74.67%, these values were determined as 74.89, 78.12 and 79.55% after 6 months of storage at 22, 28 and 35 ^{o}C, respectively (Table 2).

Brix values increased as the storage temperature increased. It is thought that this situation is due to the moisture loss of the delight samples depending on the storage conditions. Analysis of variance data in Turkish delight showed that storage temperature, time and storage temperature-time interaction were statistically significant on SSC ($p<0.05$). The changes in the color parameters as a result of the storage of the samples prepared with the addition of crab apple juice concentrate at different temperatures and times are given in Table 3. Initially, the brightness value of Turkish delight samples colored with crab apple juice concentrate is 30.56. It was determined that there was no significant change in *L** value after 6 months of storage at 22 and 28 ^{o}C. The *L** value decreased to 29.33 at the end of 6 months in the samples stored at 35 ^{o}C. This decrease in *L** values may be related to browning of Turkish delight samples and pigment degradation. Batu, Arslan [19] in their study similarly stated that thermal decomposition of anthocyanins in Turkish delight increased browning and darkened the color of Turkish delight. The initial *a** value of Turkish delight was determined as

2.96. As the storage temperature and time increased, the *a** value in the colors of delights, that is, the redness decreased. The highest *a** value was (3.57) in the samples stored at 22 °C for 1 month, and the lowest value (-0.66) was determined in the samples stored at 35 °C for 6 months. The color of rose flavored Turkish delight (RFTD) samples stored at 22 °C were better preserved than those stored at 28 and 35 °C. According to a study on Turkish delights colored with black grape and cherry concentrates, *a** value increased as the total anthocyanin content increased [19]. While the *b** value initially measured in Turkish delight samples was -0.79, it generally increased with the increase in storage temperature and time. The *b** value in Turkish delights varied between 0.62-1.60. The *b** value of Sultan Turkish delight samples produced with the addition of 17-19% pomegranate seeds was initially measured as 7.61. The *b** values of the samples stored at room temperature were 8.45 at the 2nd month, 9.26 at the 4th month, and 9.18 at the 6th month [21]. The *C** value, which was 3.09 at the beginning, decreased to 1.50, 1.37 and 1.32 after 6 months of storage at 22, 28 and 35 °C, respectively. The *h* value of the RFTD samples, which was measured at the beginning, decreased after 1 month of storage at 22 °C, but started to increase again as the storage temperature and time increased. The highest result was determined in samples (121.30) stored at 35 °C for 6 months. The effects of storage temperature and storage time, which are sources of variance, on *L**, *a**, *b**, *C** and *h* values were found to be statistically significant ($p<0.05$). While the effect of interaction on *L**, *C** and *h* values was statistically significant, its effect on *a** and *b** values was insignificant. While the *L** value of the uncolored and unflavored Turkish delight produced is 40.07, the *a** value is -4.08 and the *b** value is 2.51, the color parameters of the Turkish delight enriched with 7.5% sour cherry juice and black grape concentrate (65 brix) were measured as 24.61, 1.84, 2.93 and 26.10, 4.12, 2.70, respectively [19]. Ozen and coauthors [12] color measurement values (*L**, *a**, *b**, *C** and *h*) in Turkish delight colored with the addition of black carrot anthocyanins were initially measured as 26.02, 6.4, -0.28, 6.4 and 357.46, respectively. Samples were stored at 12, 20 and 30 °C for 150 days. At the end of storage, the color parameters changed to 26.19, 5.3, -0.94, 5.4, 349.96 at 12 °C, 26.53, 5.8, -1.20, 5.9 and 348.39 at 20 °C, and 27.13, 4.8, -0.72, 4.8 and 350.15 at 30 °C. In another study, the *L**, *a** and *b** values of Turkish delight samples to which cranberry pulp was added at a concentration of 4.4% were 36, 12.5 and 8.2, respectively. These values were found to be 47.3, 10.1, 7.5 after 10 weeks at 18-25 °C and 48.3, 9.4, 7.8 after 20 weeks of storage. The *L**, *a** and *b** values of Turkish delight samples supplemented with 12.2% cranberry pulp were 30.67, 14.10 and 7.3, respectively. It was determined as 41.3, 7.6, 5 after 10 weeks at 18-25 °C and 48.5, 7.2, 5.3 after 20 weeks of storage [20]. The color parameters of Turkish delight produced with different raw materials and the values we obtained in our study differed. However, the changes in color parameters were similar with the increase in storage temperature and time.

Table 2. pH, TA and brix values of Turkish delight samples stored at different temperatures and periods.

Temperature (°C)	Storage Period (day)	pH	TA (g/100g)	SSC (%)
22	30	3.28±0.04	0.22±0.01	78.79±0.54^{a-d}
	60	3.37±0.02	0.23±0.01	76.00±1.17^{def}
	90	3.33±0.00	0.21±0.01	79.76±0.36^{abc}
	120	3.34±0.01	0.22±0.00	75.42±0.00^{ef}
	150	3.32±0.01	0.22±0.00	76.38±0.58^{def}
	180	3.36±0.03	0.22±0.00	74.89±0.46^{f}
28	30	3.33±0.06	0.21±0.01	80.63±0.14^{ab}
	60	3.34±0.04	0.23±0.00	76.08±1.14^{def}
	90	3.32±0.02	0.21±0.01	79.93±0.96^{abc}
	120	3.30±0.01	0.22±0.00	75.33±0.97^{ef}
	150	3.34±0.01	0.21±0.00	75.68±0.24^{ef}
	180	3.36±0.01	0.21±0.00	78.12±0.59^{b-e}
35	30	3.33±0.01	0.21±0.00	79.53±1.07^{abc}
	60	3.33±0.00	0.23±0.00	77.64±0.20^{c-f}
	90	3.34±0.00	0.21±0.02	81.08±1.14^{a}
	120	3.37±0.01	0.21±0.00	76.51±0.67^{def}
	150	3.33±0.00	0.21±0.00	78.70±0.50^{a-d}
	180	3.39±0.03	0.21±0.01	79.55±0.51^{abc}

Table 3. Color parameters (*L**, *a**, *b**, *C**, *h*) of Turkish delight samples stored at different temperatures and periods

Storage temperature (°C)	Storage Period (day)	*L**	*a**	*b**	*C**	*h*
22	30	32.68±0.39^{ab}	3.57±0.399	0.62±0.022	3.62±0.43^{a}	8.93±1.74^{i}
	60	32.75±0.16^{a}	3.10±0.17	0.76±0.28	3.25±0.17^{ab}	17.99±0.29^{hi}
	90	32.09±0.60^{abc}	2.22±0.03	0.92±0.14	2.39±0.11^{bcd}	20.23±2.67^{hi}
	120	32.30±0.95^{abc}	1.68±0.00	0.74±0.09	1.88±0.01^{cde}	21.32±0.25^{hi}
	150	32.67±0.13^{ab}	1.52±0.10	0.70±0.12	1.55±0.14^{de}	28.89±0.80^{fgh}
	180	32.88±0.34^{a}	1.65±0.13	1.40±0.03	1.50±0.04^{de}	41.28±1.09^{efg}
28	30	32.86±0.19^{a}	3.01±0.12	1.02±0.02	3.19±0.12^{ab}	18.48±0.13^{hi}
	60	32.72±0.74^{a}	2.29±0.49	1.50±0.50	2.71±0.72^{abc}	31.06±5.32^{fgh}
	90	31.70±0.21^{abc}	1.07±0.23	1.23±0.21	1.72±0.20^{cde}	45.25±4.74^{ef}
	120	32.20±0.78^{abc}	0.81±008	1.14±0.13	1.62±0.21^{de}	54.82±0.29^{de}
	150	32.50±0.31^{abc}	0.64±0.16	1.40±0.08	1.38±0.37^{e}	68.65±8.01^{cd}
	180	32.28±0.34^{abc}	0.49±0.12	1.60±0.55	1.37±0.07^{e}	79.95±1.73^{bc}
35	30	32.02±0.51^{abc}	1.52±0.02	0.75±0.07	1.79±0.11^{cde}	27.66±2.12^{gh}
	60	32.26±0.31^{abc}	0.78±0.36	1.53±0.16	1.69±0.20^{de}	67.56±0.70^{cd}
	90	31.80±0.74^{abc}	0.15±0.02	1.60±0.05	1.55±0.13^{de}	87.26±3.50^{b}
	120	30.31±0.58^{cd}	-0.45±0.18	1.50±0.07	1.46±0.03^{de}	112.93±12.85^{a}
	150	30.43±1.23^{bcd}	-0.59±0.08	1.39±0.08	1.38±0.06^{e}	120.26±3.59^{a}
	180	29.33±0.07^{d}	-0.66±0.08	1.33±0.22	1.32±0.01^{e}	121.30±0.10^{a}

Changes in total phenolic compound and antioxidant activity during storage

TP compounds of Turkish delight was found to be 542.54 mg GAE/kg. The increase in storage time caused a decrease in the amount of TP substances in Turkish delight samples stored at 22 and 28 °C. The highest analysis results were found in samples stored for 1 month at 22 °C (538.90 mg GAE/kg) and 28 °C (540.42 mg GAE/kg). In the samples stored at 35 °C, a fluctuation was occurred in the amounts of phenolic compounds after the first two months of storage. The lowest amount of TP substance was found in samples stored at 35 °C for 2 months with 453.21 mg GAE/kg (Table 4). The Folin-Ciocalteu method is accepted as an antioxidant activity determination method as well as being a TP substance determination method. Maillard reaction products, which are formed during storage and exhibit antioxidant activity, react with the Folin-Ciocalteu reagent. For this reason, high values can be determined in the results of TP substance analysis with the Folin-Ciocalteu method in products that have been heat treated or stored at high temperatures. The effects of storage temperature and storage time on the TP content of Turkish delight were statistically insignificant ($p>0.05$). The effect of storage temperature-time interaction on the total amount of phenolic substance was found to be statistically significant ($p<0.05$). Black grape and cherry concentrates with 65% dry matter were added to uncolored and unflavored Turkish delight separately in concentrations of 2.5%, 5.0% and 7.5% by mass. Depending on the added black grape concentration, the TP content values were found as 263.97, 271.45 and 292.02 µg pyrocatechol/mg, respectively. Depending on the fruit concentration, the total amount of phenolic substances in the sour cherry concentrated Turkish delight was found to be 262.11, 279.58 and 291.55 µg pyrocatechol/mg [19]. ABTS value was found as 2.98 mmol TE/kg at the beginning of storage in the concentrated added Turkish delight samples. ABTS values increased and decreased during the selected storage temperatures and periods. According to ABTS method results, antioxidant activity levels detected in RFTD samples were found to be in the range of 2.31-2.91 mmol TE/kg (Table 4). The effects of storage temperature and storage time on the antioxidant activity results determined by ABTS method were found to be statistically insignificant ($p>0.05$). The effect of storage temperature-time interaction on ABTS value was statistically significant ($p<0.05$). According to Tukey's test results, ABTS values of Turkish delight samples stored at 22 and 28 °C were not different from each other. It was observed that the DPPH value, which was initially found as 3.43 mmol TE/kg in the Turkish delight samples, tended to decrease as the storage temperature increased and the storage time extended. Antioxidant activity of Turkish delight samples was found in the range of 3.05-3.36 mmol TE/kg according to DPPH method for 6 months storage period at 22, 28 and 35 °C. According to these results, the highest antioxidant activity was detected in the samples stored at 22 °C for 1 month, and the lowest in the samples stored at 35 °C for 6 months (Table 4). The effects of storage temperature and storage time on the antioxidant activity results determined by the

DPPH method in Turkish delight were found to be statistically significant ($p<0.05$). The statistical significance of the effects of storage temperature-time interactions on DPPH values was found to be insignificant ($p>0.05$).

Table 4. TPC, ABTS and DPPH results of Turkish delight samples stored at different storage temperature and period

Storage Temperature (°C)	Storage Period (day)	TPC (mg GAE/kg)	ABTS (mmol TE/kg)	DPPH (mmol TE/ kg)
22	30	538.90±13.661^{ab}	2.64±0.02^{ab}	3.36±0.07
	60	520.87±23.059^{abc}	2.72±0.11^{ab}	3.23±0.01
	90	513.86±5.120^{abc}	2.65±0.10^{ab}	3.18±0.01
	120	511.80±11.080^{abc}	2.74±0.17^{ab}	3.21±0.02
	150	510.38±35.136^{abc}	2.78±0.08^{ab}	3.14±0.10
	180	507.27±12.127^{abc}	2.61±0.07^{ab}	3.06±0.01
28	30	540.42±16.044^{ab}	2.79±0.20^{ab}	3.30±0.04
	60	508.53±12.997^{abc}	2.63±0.03^{ab}	3.18±0.06
	90	505.94±39.902^{abc}	2.60±0.13^{ab}	3.18±0.06
	120	502.97±4.716^{abc}	2.73±0.04^{ab}	3.19±0.01
	150	477.32±21.645^{bc}	2.73±0.04^{ab}	3.08±0.05
	180	461.03±1.442^{bc}	2.70±0.39^{ab}	3.05±0.06
35	30	475.60±41.966^{bc}	2.76±0.01^{ab}	3.22±0.03
	60	453.21±16.921^{c}	2.72±0.16^{ab}	3.17±0.03
	90	506.68±0.671^{abc}	2.54±0.01^{ab}	3.09±0.04
	120	499.29±16.080^{abc}	2.31±0.01^{b}	3.06±0.01
	150	502.07±10.755^{abc}	2.45±0.06^{ab}	3.06±0.04
	180	564.21±3.585^{a}	2.91±0.15^{a}	3.05±0.03

Batu and Arslan [19] reported that the antioxidant activity (% inhibition) of uncolored and unflavored Turkish delight they produced was 6.40. Antioxidant activity values were determined as 72.65, 74.39 and 86.11 in Turkish delights enriched with 2.5, 5 and 7.5% sour cherry juice concentrate (65 brix), and 28.09, 36.40 and 48.21 in Turkish delights enriched with black grape juice concentrate at the same rates.

Changes in total monomeric anthocyanin during storage and its degradation kinetic parameters

Storage temperature and time are the main factors that cause anthocyanin losses [24]. The initial anthocyanin content of Turkish delight produced by adding crab apple juice concentrate was found to be 6.49 mg/kg. After 6 months of storage of Turkish delight, total anthocyanin losses were determined as 53.15% at 22 ^{o}C and 80.12% at 28 ^{o}C. Anthocyanin amounts were determined depending on the storage period (60, 90, 120 and 150 days) in Turkish delight samples colored with black carrot anthocyanins stored at different temperatures (12, 20 and 30 ^{o}C). The initial anthocyanin amount was found to be 12.62 mg/kg. The results were 8.37 mg/kg, 7.35 mg/kg, 5.46 mg/kg and 3.69 mg/kg, respectively, at storage times at 12 ^{o}C. 8.77 mg/kg, 8.32 mg/kg, 6.34 mg/kg and 5.27 mg/kg at the end of storage periods at 20 ^{o}C. At 30 ^{o}C, there was a decrease up to 8.05 mg/kg, 6.65 mg/kg, 4.55 mg/kg and 3.05 mg/kg levels [12]. Anthocyanins show antioxidant activity in relation to their molecular structures and redox properties [25]. The decrease in antioxidant activity values with the increase in storage temperature and time in the samples supports this situation. In addition, since anthocyanins are in the phenolic substance group, the total amount of anthocyanins decreased as the total phenolic compounds were lost during storage [26]. This situation was compatible with the reductions in the values we obtained in our current study. The reaction rate constants (k) were determined by using the values obtained as a result of the TMA amount analysis of the samples during storage. Based on the reaction rate constants, the half-life ($t_{1/2}$), activation energy (E_a), temperature quotient (Q_{10}) and z values of the samples were calculated. The changes in the amount of anthocyanin as a result of storing the samples colored with crab apple juice concentrate at different temperatures are given in Figure 1. 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 [8, 27].

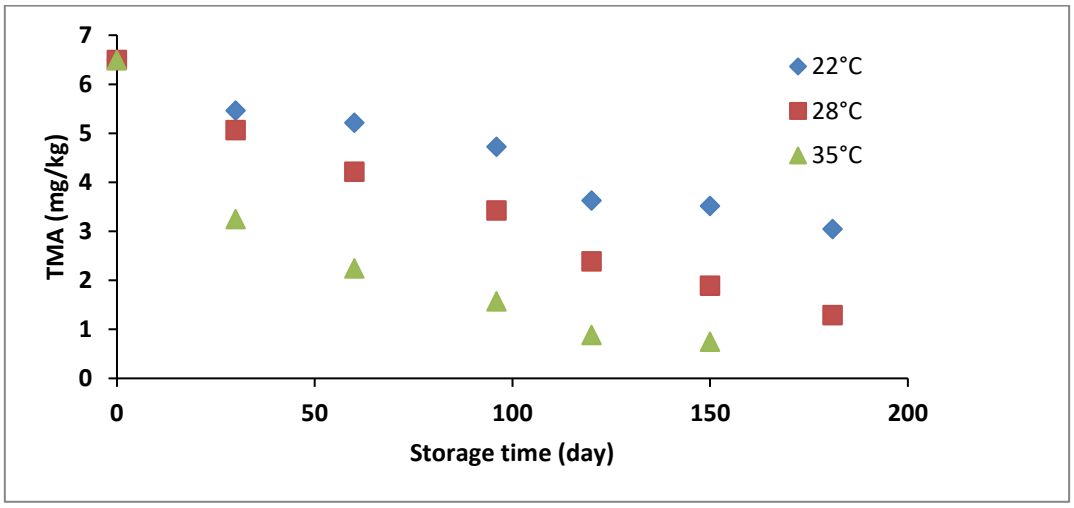

Figure 1. Changes in anthocyanin content during storage of Turkish delight samples colored with crab apple concentrate at different temperatures.

The kinetic parameters of the degradation of anthocyanins as a result of the storage of Turkish delight with crab apple juice concentrate at 22, 28 and 35 °C are shown in Table 5. When the kinetic values are examined, it is seen that the degradation rates of anthocyanins increase as the storage temperature and time increase, as expected. The rate constants were determined as $-4.2x10^{-3}$ day^{-1}, $-8.7x10^{-3}$ day^{-1} and $-14.2x10^{-3}$ day^{-1} in Turkish delight samples stored at 22, 28 and 35 °C, respectively. Looking at the half-life of the samples in Table 5, it is seen that it decreases with the increase in storage temperature. The half-life of anthocyanins in Turkish delight stored at 22, 28 and 35 °C were determined as 165.04, 79.67 and 48.81 days, respectively. When compare the results of half-life obtained at 22 and 35 °C, approximately one and a half times increase in storage temperature caused nearly a three and a half times shortness of half-life of anthocyanins in delights. The higher the activation energy, the more sensitive the reaction rate to temperature change. The activation energy in samples was determined as 70.53 kJ mol^{-1}. The Q_{10} value is a coefficient used to describe the dependence of the reaction rate on temperature. Table 5 shows how many times the reaction rates of the samples increase with temperature increase between 22-28 °C, 28-35 °C and 22-35 °C. The average value in all the given temperature ranges was determined as 2.64. The higher the z value, the less the reaction is affected by temperature changes. The z value was calculated as 24.67 °C. In the 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. The optimum storage temperature for Turkish delights colored with black carrot anthocyanins has been reported as 20 °C [12]. No study has been found in the literature on the storage stability of crab apple anthocyanins in food products. In addition, studies on the degradation kinetics of anthocyanins in Turkish delight samples are very limited. 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 °C. The rate constants of crab apple anthocyanins at 70, 80 and 90 °C 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} [28].

Table 5. Kinetic parameters for the degradation of anthocyanins after storage of Turkish delight samples

Temperature (°C)	-k *x* 10^{-3} (day^{-1})	$t_{1/2}$ (day)	E_a (kJ mol^{-1})	z (°C)	Q_{10}			
					(22-28)	(28-35)	(22-35)	Average value
22	4.2 (0.9627)*	165.04	70.53 (0.9757)*	24.67	3.37	2.01	2.55	2.64
28	8.7 (0.9823)	79.67						
35	14.2 (0.9758)	48.81						

*R^2 value

In the study investigating the storage stability of black carrot anthocyanins in rose delight, the rate constant for the degradation of black carrot anthocyanins at 20 °C was found to be $-4.61x10^{-3}$ day^{-1}. When the storage temperature increased to 30 °C, the rate constant increased to $-9.21x10^{-3}$ day^{-1}. The rate constant at 12 °C was -6.91 $x10^{-3}$ day^{-1}. It was determined that the Q_{10} value between two storage temperatures of 20 and 30 °C was approximately 2. The half-life ($t_{1/2}$) was 100 days in Turkish delights stored at 12 °C, 150 days at 20 °C and 75 days at 30 °C [12]. When these values are compared with the kinetic values obtained from

rose delight colored with crab apple anthocyanins, it is seen that the results are not very different from each other. However, the initial anthocyanin amount of the samples, the composition of the different anthocyanin source used, and the different storage temperature-times may be the factors in the emergence of different results.

CONCLUSION

The production of anthocyanins as natural food colorants and their use in the food industry are becoming widespread. 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 (*Malus floribunda*) anthocyanins be used as a new natural colorant source in Turkish delight production. At the same time, thanks to the antioxidant activity of crab apple fruit, it provides functional properties to Turkish delight. This study will be beneficial in terms of evaluating crab apples, which are not suitable for consumption as fresh, as a natural colorant source in the food industry, enriching Turkish delight, one of our traditional foods, and providing added value to the economy.

Funding: "This research was funded by SELCUK UNIVERSITY SCIENTIFIC RESEARCH PROJECTS COORDINATORSHIP (SÜBAP), grant number 17201128".
Conflicts of Interest: "The authors declare no conflict of interest."

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