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Enhancement of Strawberry Marmalade with Crab Apple (*Malus floribunda)* Anthocyanins

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Highlights:

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

ABSTRACT:

The usability of crab apple (*Malus floribunda*) as a natural colorant and anthocyanin source to stabilize the color of strawberry marmalades was investigated. Crab apple juice concentrate was added to strawberry marmalades at a rate of 2% during the production phase. The pH values of the samples changed in the range of 3.36-3.46 during the 6-month storage periods at 9, 22, and 35 ºC. With the increase in storage temperature and time, the titration acidity (TA) decreased compared to the initial values. Soluble solid content (SSC) tended to decrease with increasing storage temperature and time. As the storage temperature and time increased, the L*, a*, b* and C* values of the samples decreased, while the h values increased. The increase in temperature and time in the 6-month storage period caused a significant decrease in total phenolic content (TPC), total monomeric anthocyanin (TMA) and antioxidant activity values. The losses in the amount of TMA were measured as 36.53%, 70.74% and 91.46% in the samples stored at 9, 22, and 35 ºC, respectively. According to the kinetic data, the degradation of crab apple anthocyanins occurred according to first-order reaction kinetics during storage. The rate constants of the samples stored at 9, 22, and 35 ºC were determined as 2.6×10^{-3} day^{-1}, 6.5×10^{-3} day^{-1} and 12.9×10^{-3} day^{-1}, respectively. The addition of crab apple juice concentrate to strawberry marmalades increased the color intensity and anthocyanin stability that decreased during production and storage, and made the product functional.

Keywords:

- Anthocyanin
- Storage
- strawberry marmalade
- color
- crab apple

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INTRODUCTION

Strawberries (*Fragaria ananassa*) contain nutritive compounds (vitamins, minerals and dietary fiber) and polyphenolic phytochemicals (anthocyanins, phenolic acids and tannins) (Abdel-Hady et al., 2014; Bingöl et al., 2022). Strawberry is one of the most preferred raw materials in jams and marmalades produced worldwide (Kovacevic et al., 2015). The attractive and stable color of strawberry jam and marmalade is an important quality criterion that affects consumer preferences (Kırca et al., 2007; Martinsen et al., 2020). Colorants are used in the food industry for the purposes of preserving the original appearance of the food, ensuring the color uniformity of the product and giving color to the colorless foods. Food colorants are generally divided into three basic classes as synthetic, nature-identical and natural (Ngamwonglumlert et al., 2017; Albuquerque et al., 2020). Synthetic colorants have advantages such as low cost, ease of application and more 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 (Pinela et al., 2019; Cervera-Chiner et al., 2021).

Anthocyanins, which are natural color pigments, as well as their ability to color foods, have antioxidant and bioactive properties that are linked to certain health benefits such as anti-diabetic, anti-inflammatory and anti-cancer effects (Rodriguez-Amaya, 2016). Anthocyanins, responsible for the red color of strawberries, are the most abundant flavonoid compounds in strawberries (Kovacevic et al., 2015). However, the anthocyanins found in strawberries are unstable and susceptible to degradation during processing and storage (Kovacevic et al., 2015; Bingöl et al., 2022). External environmental conditions such as pH, temperature, light, oxygen, enzymes and presence of metal ions affect the stability of anthocyanins (Castaneda-Ovando et al., 2009; Fang et al., 2020). Therefore, the color of strawberry products needs to be enhanced with natural colorants or other sources of anthocyanins (Giusti and Wrolstad, 2003; Kırca et al., 2007). The negative effects of high storage temperatures and long storage time on anthocyanin content and color in strawberry jam and marmalade have been reported in studies (Kırca et al., 2007; Kopjar et al., 2009; Martinsen et al., 2020). However, no study has been found in the literature on the enhancing of strawberry marmalades with crab apple anthocyanins and investigating their storage stability.

In this study, it was aimed to evaluate the intense red colored crab apple (*Malus floribunda*) fruit, whose peel and flesh contain abundant phenolic compounds (Coklar et al., 2018), as an anthocyanin source. The produced crab apple juice concentrate (70 ºBrix) was added to the strawberry marmalade samples during production. The storage stability and changes in some physicochemical parameters of the samples taken every month during the 6-month storage period at three different temperatures (9, 22 and 35 ºC) were investigated.

MATERIALS AND METHODS

Material

Crab apple was collected from trees planted for landscaping from Selcuk University Alaeddin Keykubat Campus (Konya, Turkey, GPS coordinates: 38° 1′ 12.3312" and 32° 30′ 52.2252") in 2019.

Methods

Crab apple juice concentrate and strawberry marmalade production

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 SSC of 70 under vacuum at 50 ºC. The concentrate was stored at -18 ºC until

to marmalade production. Strawberries were obtained from a local producer in Alanyurt village of Aksaray province. After washing and sorting the strawberries, they were homogenized by passing through a blender (Arçelik K-1260 hand blender with grater). 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 ºC, citric acid solution (50% w/v) was added to adjusted to pH 3.2. Heat treatment was applied until the brix was 66% and set to 101 ºC final temperature. Crab apple juice concentrate (2%) was added to marmalades. After cooling to the 93 ºC, filled into the 200 mL of glass jars.

Storage conditions and shelf life experiments

The jars filled with strawberry marmelade were stored in incubators (Nüve Es 120 refrigerated incubator) at selected temperatures of 9, 22 and 35 ℃ for 6 months.

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

The pH and pH 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 ℃ 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 citric acid and the results were given as g citric acid equivalent 1/100 g (Cemeroğlu, 2013a). SSCs of water diluted and filtered samples were measured using a refractometer (Atago HSR-500, Japan) (Cemeroğlu, 2013a). L*, a*, b*, h and C* values of the samples were measured with Konika Minolta CM-5 model colorimeter (Konika-Minolta, Osaka, Japan).

Extraction procedure for TPC, TMA and antioxidant acitivity analyses

The extraction procedures applied in Coklar et al. (2018) were modified. A 5 g of marmalade was weighed and extracted with 10 mL of methanol and 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℃ 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, Nüve, Turkey) for 10 minutes at 7000 rpm. Then, while supernatant of non-acidified methanol was taken for the analysis of TPC and antioxidant acitivity analyses, supernatant of acidified methanol extract (5 mL) was evaporated under vacuum in an rotary evaporator to remove the methanol. The anthocyanin extract was resuspended in a 0.5 mL methanol and used in TMA analysis.

TPC analysis

To determine the TPC, 2.5 mL of 0.2 N folin-ciocalteu reagent (Merck 109001, USA) and 2 mL of sodium carbonate (Merck 106392, USA) (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 (Singleton and Rossi, 1965).

ABTS and DPPH antioxidant acitivity 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. To determine the DPPH antioxidant activity, 3.9 mL of DPPH (Merck D9132, USA) 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 (Brand-Williams et al., 1995).

TMA content analysis

The TMA contents in concentrate and strawberry marmalade samples were determined using the pH differential method described (Akbulut and Coklar, 2015). 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 1. The TMA contents of the samples were calculated according to 2 and the results were expressed in mg cyanidin-3-galactoside equivalent /kg

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

$$TMA\ (mg/L)=(A \times MW \times DF \times 1000)/(\varepsilon \times l) \quad (2)$$

Where; *MW*: Molecular weight for cyanidin-3-galactoside, *ε*: Molar extinction coefficient for cyanidin-3-galactoside (L x/mol 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 strawberry marmalade followed a first-order reaction law. First-order reaction law is defined by 3 and taking integration of 3 gives the 4 for integrated first-order reaction rate law (Cemeroğlu, 2015).

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

$$C=C_0e^{-kt} \quad (4)$$

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 5.

$$K=k_0e^{Ea/RT} \quad (5)$$

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 6.

$$Q_{10}=(k_2/k_1)^{10/(T2-T1)} \quad (6)$$

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

$$z=\ln 10\ R\ (T2-T1)/E_a \quad (7)$$

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

Statistical Analysis

For the strawberry marmelade 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 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 AND DISCUSSION

Some Properites of Crab Apple Juice Concentrate

Some properties of the concentrate obtained from the crab apple fruit used in the coloring of the strawberry marmalade samples are as follows: The pH value of crab apple juice concentrate (70 brix) used in our study was calculated as 2.90 and TA as 9.09 g /100 g. L*, a*, b*, C* and h color values were found as 14.89, 46.43, 24.86, 52.67 and 28.16, respectively. TPC and TMA amount were determined as 29.66 mg GAE (gallic acid equivalent) g^{-1} and 442.47 mg /kg, respectively. It has been stated in studies that wild apple varieties (MF) should be evaluated in food and beverages for reasons such as containing bioactive components (vitamin C, dietary fiber, antioxidants etc.) and attractive color (Yoshizawa et al., 2004; Coklar et al., 2018). There is no literature information on the use of crab apples selected for the study as an anthocyanin source in food products. Coklar et al. (2018) found pH value of *Malus floribunda coccinella* to be 2.89, TA value to 2.21, brix 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 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 dry weight, respectively. Yoshizawa et al. (2004) DPPH radical scavenging activity (% inhibition) of *Malus floribunda* fruit juice was determined as 53.2. The anthocyanin contents of crab apple juice concentrate used in this study was higher than strawberry (Garzon and Wrolstad, 2002; Sadilova et al., 2006), cherry (Sanchez et al., 2015), blackberry (Wang and Xu, 2007) and lower than black carrot (Türkyılmaz and Özkan, 2012).

Effect of concentrate addition to marmalade

The analysis results of the strawberry marmalade sample colored with crab apple juice concentrate and sample produced without the addition of concentrate are shown in Table 1. Addition of crab apple juice concentrate in strawberry 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. The addition of concentrate caused a numerical increase in antioxidant activity and TPC in strawberry marmalade. In the study, the strengthening of the color present in the strawberry marmalade samples was significantly achieved by the addition of crab apple juice concentrate.

Table 1. Some properties of colored and noncolored marmalade samples

Parameters	Non-colored Marmalade	Colored Marmalade
pH	3.53 ± 0.04	3.41 ± 0.02
TA (g /100 g)	0.72 ± 0.05	0.94 ± 0.09
SSC (%)	75.20 ± 0.46	74.57 ± 2.90
L*	12.72 ± 0.09	12.12 ± 1.06
a*	17.12 ± 1.02	17.56 ± 0.46
b*	9.84 ± 0.81	9.86 ± 0.48
C*	19.82 ± 1.26	20.42 ± 0.65
h	30.04 ± 1.06	29.05 ± 0.41
DPPH	8.21 ± 0.63	9.78 ± 0.39
ABTS	12.84 ± 0.77	15.50 ± 0.04
TPC (mg GAE g^{-1})	1269.85 ± 50.41	1504.99 ± 1.81
TMA (mg /kg)	45.43 ± 0.99	60.79 ± 6.14

Changes in pH, titratable acidity, soluble solid content and color parameters of marmalade during storage

In the production of products such as jam and marmalade, the pH level is great importance in order to provide a good gel formation and flavor (Cemeroğlu, 2013b). The pH values of the samples prepared with the addition of crab apple juice concentrate increased and decreased during 6 months of storage at

9, 22 and 35 ℃. The lowest pH value (3.36) was measured in the samples stored at 9 ℃ for 5 months, while the highest pH value (3.46) was measured in the samples stored at 35 ℃ for 3 months (Table 2.). 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 ($p>0.05$). According to the results of Tukey's test, it was determined that the pH values of strawberry marmalades stored at all three temperatures were not different from each other.

Table 2. pH, TA (g/100 g) and SSC (%) values of strawberry marmalades stored at different temperatures and durations

Storage Temperature (°C)	Storage Period (day)	pH	TA (g /100 g)	SSC (%)
9	30	3.37 ± 0.02	0.90 ± 0.04	76.26 ± 3.77
	60	3.40 ± 0.01	0.89 ± 0.04	67.72 ± 4.75
	90	3.40 ± 0.01	0.77 ± 0.04	64.05 ± 1.89
	120	3.40 ± 0.02	0.86 ± 0.03	64.32 ± 1.30
	150	3.36 ± 0.01	0.86 ± 0.04	67.28 ± 2.41
	180	3.39 ± 0.03	0.86 ± 0.04	68.88 ± 2.44
22	30	3.40 ± 0.01	0.89 ± 0.04	74.27 ± 0.19
	60	3.41 ± 0.01	0.83 ± 0.02	67.43 ± 0.56
	90	3.45 ± 0.00	0.76 ± 0.02	65.14 ± 0.80
	120	3.41 ± 0.02	0.86 ± 0.04	67.63 ± 1.86
	150	3.37 ± 0.02	0.83 ± 0.04	65.12 ± 1.40
	180	3.38 ± 0.02	0.84 ± 0.05	69.10 ± 1.48
35	30	3.42 ± 0.03	0.85 ± 0.03	74.12 ± 1.77
	60	3.42 ± 0.01	0.82 ± 0.02	69.44 ± 4.16
	90	3.46 ± 0.02	0.70 ± 0.04	63.35 ± 1.49
	120	3.45 ± 0.00	0.81 ± 0.02	66.10 ± 1.01
	150	3.40 ± 0.01	0.81 ± 0.04	66.64 ± 1.94
	180	3.38 ± 0.03	0.80 ± 0.03	66.84 ± 1.06

Cervera-Chiner et al. (2021) reported the pH of the traditional strawberry jam samples they produced as 3.45. Martinsen et al. (2020) found the pH value of strawberry jams produced at 93 ℃ to be 3.22. In another study, the initial pH value of strawberry jam samples was given as 3.62. The pH values at 2, 4 and 6 months were respectively 3.58, 3.60 and 3.61 in samples stored at 10 ℃, 3.63, 3.64 and 3.57 in samples stored at 20 ℃, and 3.62, 3.53 and 3.60 in samples stored at 37 ℃ (Aslanova et al., 2010). Abdel-Hady et al. (2014), the initial pH of strawberry marmalade 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. While the initial pH of the strawberry marmalade samples enriched with black carrot puree (30%) was 3.5, it was determined as 3.7 in the samples stored at room temperature for 3 months and as 4.0 in those stored for 6 months. The acidity value of the jam is due to the organic acids naturally found in the fruits and the acid added while preparing the jam (Kanwal et al., 2017). Increases and decreases in TA values of strawberry marmalades were detected during storage at all three temperatures, and decreases in TA were observed with increasing storage temperature. Results were determined in the range of 0.70-0.90 g /100 g (Table 2). The effect of storage time on TA levels of the samples was statistically significant ($p<0.05$), while the effect of storage temperature and temperature-time interactions in storage was statistically insignificant ($p>0.05$). In their study, Aslanova et al. (2010) found average TA value was 0.22% in strawberry jams. García-Viguera et al. (1999) stated that the TA of strawberry jam was 1.16%. It was emphasized that there was no change in this value during 200 days of storage at 20, 30 and 37 ℃. The initial TA value of canned strawberry samples prepared with the addition of black mulberry juice concentrate was 0.51 g /100 g, and after 6 months of storage at 5, 25 and 37 ℃, 0.52, 0.53 and 0.57 g/100 g, respectively (Hepsağ and Hayoğlu, 2019). When the data in the literature are examined, it is

thought that the differences seen according to the values we obtained in our study are due to factors such as production technique (traditional method/industrial type), citric acid ratio, 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.). The SSC values of the strawberry marmalades that produced were determined in the range of 63.35%-76.26%. While the initial SSC value of strawberry marmalades with concentrate was 74.57%, these values were measured as 68.88%, 69.10% and 66.84%, respectively, after 6 months of storage at 9, 22 and 35 ℃. Analysis of variance data showed that storage time had a statistically significant effect on SSC ($p<0.05$), while storage temperature and storage temperature-time interaction had no effect ($p>0.05$). Cervera-Chiner et al. (2021) recorded the brix value of traditional strawberry jam samples as 46.5. The brix of strawberry and raspberry jams produced at 93 ℃ were measured as 50.8% and 52.0%, respectively (Martinsen et al., 2020). It is seen that the brix values of the produced strawberry jam samples are lower than the results of the strawberry marmalade samples obtained in our study. Aslanova et al. (2010) determined the starting brix of strawberry jam samples as 72.70. The brix values of the samples stored at 10 ℃ for 2, 4 and 6 months were 72.74, 73.17 and 72.58, respectively. These values changed as 72.93, 73.82 and 73.70 at 20 ℃, and as 73.05, 72.32 and 73.08 at 37 ℃. When the initial L* values of strawberry marmalades were examined, similar results were obtained in the samples with and without concentrated addition. L* values of strawberry marmalades stored at 9, 22 and 35 ℃ for 6 months generally decreased as the temperature and time increased. L* values were determined in the range of 8.04-17.48 during storage (Table 3). It has been stated in studies that the color of the samples darkened with the decrease in L* values used as browning index in foods (Wicklund et al., 2005; Mazur et al., 2014; Martinsen et al., 2020). a* values in strawberry marmalades tended to decrease with increasing storage temperature and time. Initially, the a* value of strawberry marmalades without concentrate was 17.12, and 17.56 in the samples with concentrate added. The a* values of strawberry marmalades were determined the highest in the samples stored at 9 ℃ for 1 month (23.76), and the lowest in the samples stored at 35 ℃ for 6 months (6.95).

Table 3. L*, a*, b*, C* and h values of strawberry marmalade samples stored at different temperatures and times

Storage Temperature (°C)	Storage Period (day)	L*	a*	b*	C*	h
9	30	17.37 ± 0.51	23.76 ± 0.94	14.94 ±1.21	28.08 ± 1.45	31.88 ±0.71^{de}
	60	13.76 ± 0.56	18.30 ± 0.45	10.82 ±0.47	21.27 ± 0.16	31.23 ±0.82^{de}
	90	11.94 ± 0.75	16.79 ± 0.81	9.70 ± 0.69	19.39 ± 1.05	30.63 ±0.37^{e}
	120	11.95 ± 1.12	16.09 ± 1.03	9.71 ± 0.57	18.79 ± 1.17	30.39 ±0.91^{e}
	150	11.61 ± 0.69	15.84 ± 0.90	9.58 ± 0.38	18.52 ± 0.96	30.99 ±0.20^{e}
	180	11.69 ± 0.15	15.62 ± 0.59	9.43 ± 0.83	18.26 ± 0.07	31.12 ± 3.20^{de}
22	30	17.48 ± 0.78	21.72 ± 0.30	14.29 ± 0.35	26.04 ± 0.49	32.97 ±0.25^{cde}
	60	14.58 ± 2.46	15.43 ± 1.02	9.50 ± 1.88	18.05 ± 1.90	31.41 ±3.08^{de}
	90	11.57 ± 0.84	14.34 ± 0.97	9.44 ± 0.84	17.21 ± 1.22	33.55 ± 0.25^{cde}
	120	12.11 ± 0.81	13.99 ± 0.77	9.77 ± 0.41	17.18 ± 1.01	35.43 ±0.27^{b-e}
	150	11.71 ± 0.73	13.16 ± 1.52	9.70 ± 0.57	16.74 ± 1.55	38.14 ±1.65^{abc}
	180	11.98 ± 0.09	13.36 ± 0.83	9.78 ± 0.04	16.69 ± 0.55	36.85 ±2.19^{a-d}
35	30	16.77 ± 0.77	18.42 ± 1.19	14.33 ± 1.53	23.34 ± 1.87	37.86 ±1.14^{abc}
	60	13.61 ± 1.95	13.46 ± 1.45	11.11 ± 1.32	17.46 ± 1.96	39.49 ±0.31^{ab}
	90	10.01 ± 1.10	10.21 ± 1.36	8.82 ± 1.12	13.49 ± 1.76	40.83 ±0.15^{ab}
	120	10.44 ± 1.51	9.42 ± 1.64	8.41 ± 2.14	12.70 ± 2.57	41.87 ±1.81^{a}
	150	8.04 ± 1.32	7.29 ± 1.45	6.21 ± 1.73	9.58 ± 2.20	40.82 ±1.35^{ab}
	180	8.48 ± 0.86	6.95 ± 0.95	6.32 ± 1.02	9.44 ± 1.46	42.35 ±0.86^{a}

The decrease in a* values is due to the degradation of anthocyanins depending on the increase in storage temperature and time. The a* value decreased at the end of storage at different temperatures in strawberry jams (Wicklund et al., 2005), pomegranate jams (Melgarejo et al., 2011) and strawberry marmalades colored with black carrot anthocyanins (Kırca et al., 2007; Abdel-Hady et al., 2014). The b* value generally decreased as the storage temperature and time increased. While the initial b* values in strawberry marmalades with and without concentrate were 9.86 and 9.84, respectively, they varied between 6.21-14.94 during storage. The C* value of the concentrated added samples was initially found to be 20.42. It varied between 9.44-28.08 in strawberry marmalades stored at 9, 22 and 35 ℃, and this value decreased in general with the increase in storage temperature and time. Storage of strawberry and raspberry jams at 23 ℃ caused a paler color and decreased C* value compared to storage at 4 ℃ (Martinsen et al., 2020). The h value in strawberry marmalades was determined as 29.05 and 30.04 in products with and without concentrate added, respectively. The h value increased with the increase in storage temperature and time, and the highest value was detected in the samples stored at 35 ℃ for 6 months. The effects of storage temperature and storage time on L*, a*, b*, C* and h values were found to be statistically significant ($p<0.05$). While the effect of interaction on L*, a*, b* and C* values was insignificant, its effect on h value was significant.Initially, L*, a* and b* values of the produced strawberry jam samples were 18.3, 28.6 and 8.7, respectively. It changed to 17.0, 20.5 and 8.3 at the end of 28 days of storage at 4 ℃ and 16.5, 20.8 and 8.1 at the end of 28 days of storage at 15 ℃ (Patras et al., 2011). L*, a* and b* values were 19.68, 5.86 and 1.67, respectively in strawberry marmalades with black carrot concentrate added stored at 10 ℃ after 30 days. These values are stated as 19.66, 5.38 and 1.59 at 22 ℃ and as 19.98, 4.14 and 1.94 at 37 ℃. At the end of 120 days of storage at 10 ℃, L*, a* and b* values were determined as 20.18, 5.80 and 1.97, respectively, while 20.13, 4.93 and 2.00 at 22 ℃, and 9.68, 2.56 and 2.30 at 37 ℃ (Kırca et al., 2007).

Changes in TPC and antioxidant activity during storage

Although there were some fluctuations in the TPC amounts of the samples during storage, the increase in temperature and time caused a decrease in the TPC of the samples. It is thought that phenolic substances may be decomposed due to storage.

Table 4. TPC, ABTS and DPPH analysis of strawberry marmalade samples stored at different temperatures and times

Storage temperature (°C)	Storage Period (day)	TPC (mg GAE /kg)	ABTS (mmol TE /kg)	DPPH (mmol TE /kg)
9	30	1584.26 ± 11.08^{a}	15.26 ± 1.46	9.62 ± 0.19
	60	1425.21 ± 21.17^{bcd}	15.21 ± 0.19	9.41 ±0.61
	90	1474.82 ± 7.05^{b}	14.94 ± 0.61	9.53± 0.30
	120	1410.19 ± 13.87^{cd}	14.85 ± 1.00	9.39 ± 0.36
	150	1396.41 ± 5.06^{de}	14.62 ± 0.25	9.39 ± 0.47
	180	1407.44 ± 16.85^{cd}	14.53 ± 1.12	9.18 ± 0.91
22	30	1532.31 ± 4.04^{a}	15.07 ± 0.13	9.52 ± 0.33
	60	1284.61 ± 3.06^{gh}	14.10± 0.17	8.85 ± 0.09
	90	1455.88 ± 22.11^{bc}	14.03 ± 0.09	8.98 ± 0.18
	120	1347.40 ± 14.00^{ef}	13.65 ± 0.88	8.92 ± 0.36
	150	1198.33 ± 12.06^{jk}	13.93 ± 1.80	8.57± 0.37
	180	1174.72 ± 15.10^{k}	12.17 ± 0.30	8.69 ± 0.45
35	30	1318.37 ± 17.15^{fg}	12.93 ± 0.97	8.85 ± 0.57
	60	1245.93 ± 15.14^{hij}	12.27 ± 0.22	8.45 ± 0.08
	90	1319.77 ± 0.00^{fg}	11.88 ± 0.98	8.45 ± 0.41
	120	1278.18 ± 4.00^{ghı}	11.52 ± 0.16	8.09 ± 0.22
	150	1316.20 ± 27.68^{fg}	10.27 ± 0.40	7.82 ± 0.04
	180	1222.29 ± 8.96^{ijk}	10.47 ± 1.04	7.70 ± 0.72

However, the Folin-Ciocalteu method is accepted as an antioxidant activity determination method as well as being a total phenolic 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 total phenolic substance analysis with the Folin-Ciocalteu method in products that have been heat treated or stored at high temperatures. These values are not due to the increase of phenolic substances, but to the formation of the products of non-enzymatic browning reactions. It is thought that the increase in the total amount of phenolic substances in all the stored samples in some months may be related to this situation. The amount of TPC was determined in the range of 1174.72-1584.26 mg GAE /kg (Table 4). The effects of storage temperature, storage time and interaction on TPC of strawberry marmalade samples were found to be statistically significant ($p<0.05$).Ngo et al. (2007) stated the TP amount of the strawberry jams they produced as 262.1 mg GAE /100 g. In another study, the TP content of strawberry fruit was 8503.13 mg GAE /kg, and it decreased to 578.26 mg GAE /kg after jam production. This value was reported as 507.61, 487.68, 476.81, 467.75 and 455.07 mg GAE /kg, respectively, in the samples stored at 25 ℃ for 5 months and taken every month (Rababah et al., 2011). TP amounts of jams produced from different ripe strawberry species (Blink, Polka and Senga) were measured as 125, 80 and 88 mg GAE /100 g, respectively. These values were calculated as 130, 87 and 77 mg GAE /100 g at 4 ℃, 120, 88 and 66 mg GAE /100 g at 20 ℃ for jams stored for 3 months. TP amount has been reported to be 107, 140 and 100 mg GAE /100 g at 4 ℃, 103, 126 and 102 mg GAE /100 g at 20 ℃ in jams stored for 6 months (Mazur et al., 2014). Martinsen et al. (2020) determined the TP content of strawberry jams produced at 93 °C as 207 mg GAE 00 g^{-1}. Strawberry jam samples were measured at 230 and 156 mg GAE /100 g at 4 ℃, 216 and 137 mg GAE /100 g at 23 ℃ at the end of 8 and 16 weeks of storage. In another study, the initial TP amount of strawberry marmalade samples was stated as 156.8 mg /100 g. In the samples stored at room temperature, these values were measured at 138.7 mg /100 g and 114.5 mg /100 g at 3 and 6 months. While the initial TP amount of strawberry marmalade samples enriched with 30% black carrot puree was 212.5 mg /100 g, this value was determined as 204.8 mg /100 g in samples stored at room temperature for 3 months and 198.4 mg /100 g in samples stored for 6 months (Abdel-Hady et al., 2014). The TP content of strawberry jams and marmalades produced in the literature generally tended to decrease with increasing storage temperature and prolongation of the time. This situation is generally compatible with the results we obtained in our study. Mazur et al. (2014) 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 (Shinwari and Rao, 2018). The addition of crab apple juice concentrate increased the ABTS activity levels of the samples from 12.84 mmol TE /kg to 15.50 mmol TE /kg. The increase in storage temperature and time in marmalade samples significantly decreased the antioxidant activity values determined according to the ABTS method. ABTS value was 15.26 mmol TE /kg in samples stored at 9 ℃ for 1 month, and 10.27 mmol TE /kg in samples stored at 35 ℃ for 5 months (Table 4). The effects of storage temperature and storage time on the antioxidant capacity results determined by ABTS method were statistically significant ($p<0.05$), while the effect of interaction was statistically insignificant ($p>0.05$). The initial DPPH value of strawberry marmalades produced by adding concentrate was determined as 9.78 mmol TE /kg. The increase in storage temperature and time significantly decreased the antioxidant capacity results determined by the DPPH method. The highest values in marmalades were found in the samples stored at 9 ℃ for 1 month, and the lowest values were found in the samples stored at 35 ℃ for 6 months (Table 4). According to

variance analysis results, the effect of storage temperature on DPPH values is statistically significant ($p<0.05$). The effects of storage time and storage temperature-time interactions on DPPH values were found to be insignificant ($p>0.05$). Amakura et al. (2000) stated that the DPPH radical scavenging activity value of the strawberry jam samples they produced was 2.16 mg/mL. In another study, it was determined that the antioxidant capacity of strawberry jam decreased from 96.8% to 78.6% during the 28-day storage period at 4 °C, and to 77.5% at the end of storage at 15 °C (Patras et al., 2011). Rababah et al. (2011), the DPPH (% inhibition) value of strawberry fruit was 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 ℃ for 5 months and taken every month. Martinsen et al. (2020) recorded the antioxidant capacity of strawberry jams produced at 93 ℃ as 11.4 µmol TE g^{-1}. The antioxidant capacity of strawberry jam samples were measured as 12.5 and 11.6 µmol TE g^{-1} after 8 and 16 weeks of storage at 4 ℃, and 12.1 and 10.6 µmol TE g^{-1} at 23 ℃ for the same storage periods. As with the research findings on the total amount of TPC, the results of antioxidant activity also showed differences between studies.

It is thought that the reason why the results found are different may be due to the different components used in the research, the extraction applied, the analysis methods and the unit differences in the results given. However, similar to the results we obtained in our study, the antioxidant activity values of strawberry jam and marmalade produced in the literature decreased with increasing storage temperature and time.

Changes in TMA amount during storage

Storage temperature and time are the main factors that cause anthocyanin losses (Shinwari and Rao, 2018). The initial TMA levels of the concentrate free and added strawberry marmalade samples were determined as 45.43 mg /kg and 60.79 mg /kg, respectively. As seen in Figure 1, the amount of TMA of the samples decreased as the storage temperature and time increased. The losses in the amount of anthocyanins during storage for 6 months were determined as 36.53% at 9 ℃, 70.74% at 22 ℃ and 91.46% at 35 ℃. Since anthocyanins are a class of flavonoids (Jing et al., 2012), TMA content also decreased due to the reduction of phenolic compounds during storage. In the literature, it has been stated that the TMA content of strawberry jam and marmalade decreases with increasing storage temperature and time (García-Viguera et al., 1999; Ngo et al., 2007; Patras et al., 2011; Rababah et al., 2011; Martinsen et al., 2020). This situation is in parallel with the data obtained from our research results.

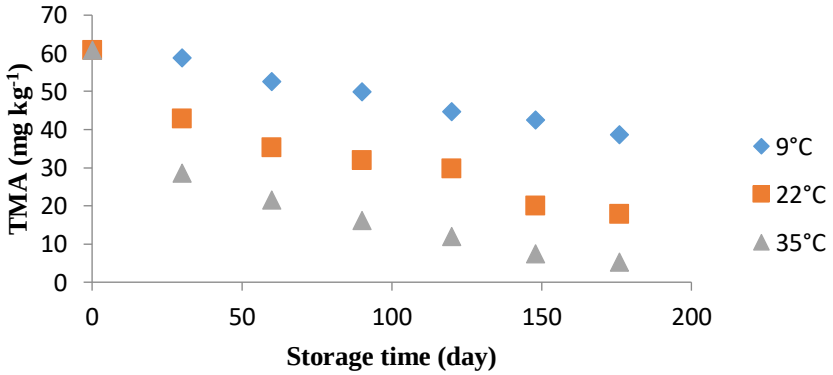

Figure 1. Changes in TMA amounts as a result of storing strawberry marmalades colored with crab apple at different temperatures

Black carrot juice concentrate was added to enhance the color of strawberry jams prepared from two types of Osmanlı and Kara. The initial anthocyanin contents of colorless jams in Osmanlı and Kara cultivars were 6.30 and 11.82 mg /kg, respectively. Anthocyanin content of the samples to which black carrot juice concentrate was added increased to 29.69 and 26.31 mg /kg in Osmanlı and Kara cultivars,

respectively. While a very rapid loss of anthocyanins occurred in a short time in marmalades stored at 37 °C, the degradation of anthocyanins was very slow in samples stored at 10°C (Kırca et al., 2007). Abdel-Hady et al. (2014), the initial TMA value of the strawberry marmalades they produced was determined as 26.7 mg /100 g. TMA values of the samples stored at room temperature were reported to be 17.8 and 12.5 mg /100 g at 3 and 6 months. The initial TMA content of strawberry marmalades enriched with 30% black carrot puree was 58.9 mg /100 g. This value was calculated as 56.4 mg /100 g for samples stored at room temperature for 3 months and as 54.6 mg /100 g for samples stored for 6 months.

Degradation kinetics

Strawberry marmalades produced with the addition of crab apple juice concentrate were stored for 6 months at different temperatures and kinetic parameters were calculated using the values obtained as a result of the TMA amount analysis of the samples. The changes in the amount of anthocyanins as a result of storing the samples colored with crab apple juice concentrate at different temperatures are given in Figures 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 (Chatham et al., 2020; Ertan et al., 2020). The kinetic parameters of the degradation of anthocyanins during storage of marmalades with added crab apple juice concentrate at 9, 22 and 35 ℃ are shown in Table 5. As the storage temperature of the samples increased, the reaction rate constants increased. The rate constants were determined as $2.6x10^{-3}$ day^{-1}, $6.5x10^{-3}$ day^{-1} and $12.9x10^{-3}$ day^{-1} in strawberry marmalades stored at 9, 22 and 35 °C, respectively. Studies have shown that as the storage temperature increases, the *k* value increases (Wang and Xu, 2007; Türkyılmaz and Özkan, 2012). 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 marmalades stored at 9, 22 and 35 °C were 266.60, 106.64 and 53.73 days, respectively. It is seen that the stability of crab apple anthocyanins during storage at 35 ℃ is at the lowest level. Other authors also reported that $t_{1/2}$ values decrease with increasing temperature (Kırca et al., 2007; Harbourne et al., 2008; Kara and Ercelebi, 2013). Activation energy (E_a) and temperature quotients (Q_{10}) values indicate the temperature dependence of anthocyanin degradation. The higher the activation energy, the more sensitive the reaction rate to temperature change. The activation energy in strawberry marmalade was determined as 44.60 kJ/mol. Table 5 shows how many times the reaction rates of the samples increase with temperature increase between 9-22 ℃, 9-35 ℃ and 22-35 ℃ for strawberry marmalades. Higher Q_{10} value indicated that anthocyanins were more sensitive to temperature elevations. Considering the average value in all temperature ranges given, it was determined as 1.86. 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.

Table 5. Kinetic parameters for the degradation of anthocyanins after storage of strawberry marmalade samples

Temperature(℃)	k$x10^{-3}$ (day^{-1})	$t_{1/2}$ (day)	Activation energy (kJ/mol)	Z (℃)	Q_{10}			
					(9-22)	(9-35)	(22-35)	Average value
9	2.6 (0.9887*)	266.60	44.60 (0.9945*)	37.37	2.02	1.85	1.69	1.86
22	6.5 (0.9603)	106.64						
35	12.9 (0.9792)	53.73						

*R^2 value

The z value of the samples was calculated as 37.37℃.

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. It has been reported in studies that strawberry jams should be stored at 4 ℃ in order to minimize anthocyanin losses (Kopjar et al., 2009; Patras et al., 2011; Martinsen et al., 2020). 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 (MF) 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 ℃. The rate constants of crab apple anthocyanins at 70, 80 and 90 ℃ 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 (Yeşil, 2018). During heating to 90, 105 and 150 ℃ temperatures, $t_{1/2}$ values were calculated as 167.2, 103.8 and 13.4 min, and k values were calculated as 4.1 x 10^{-3} min^{-1}, 6.7 x 10^{-3} min^{-1} and 51.6 x 10^{-3} min^{-1}, respectively, in strawberry juices (Bingöl et al., 2022). The k value of strawberry nectar, which was prepared by adding sucrose at a concentration of 20% and stored at 20 ℃ for 42 days, was determined as 26.5 x10^{-2} $days^{-1}$ and its half-life was 26 days (Ertan et al., 2020). The k values calculated according to the total amount of TMA in strawberry jams stored at 4 and 15 °C were determined as 0.95×10^{-2} day^{-1} and 1.71×10^{-2} day^{-1}, respectively (Patras et al., 2011). The k values in blackberry jam samples stored at 10 and 25 ℃ for 180 days were calculated as 0.0024 day^{-1} and 0.0125 day^{-1}, respectively. The Q_{10} value was determined as 3.0 and the E_a value as 19,490.33 kcal/mol (Moura et al., 2012). When the results obtained with these data are compared, it shows that the addition of crab apple juice concentrate increases the storage stability of the products. The rate constants of strawberry jams enriched with black carrot juice concentrate stored at 10, 22 and 37 ℃ were 2.53x10^{-3} day^{-1}, 4.60 x10^{-3} day^{-1} and 14.50 x10^{-3} day^{-1}, respectively. $t_{1/2}$ values were 39.1, 21.5 and 6.8 weeks, respectively (Kırca et al., 2007). These values show that the results are close to each other when compared with the rate constants for the degradation of anthocyanins in strawberry marmalades with added crab apple juice concentrate, but when the $t_{1/2}$ values are compared, black carrot anthocyanins are more stable than crab apples. It is thought to be the reason why black carrots contain acylated anthocyanins. Food matrices have a great influence on the stability of anthocyanins. Different anthocyanin sources used for enrichment in production, different storage temperatures and times are main reasons for emergence of different kinetic parameters of anthocyanins in studies (Kırca et al., 2006; Kırca et al., 2007; Ozen et al., 2011).

CONCLUSION

Due to the rapid degradation of pigments in strawberry products such as marmalade, color loss is observed during the production and storage stages. Therefore, the low stability of anthocyanins in strawberries should be enhanced with natural colorants or other anthocyanin sources. Providing the production of functional products by using natural colorants instead of synthetic colorants in food formulations will also contribute to the healthy nutrition of consumers. It is important to bring new and alternative natural colorant sources to the food industry and to investigate the stability of these sources in food products. In this study, crab apple fruits were used as a new and alternative natural source of anthocyanins for the strawberry marmalades produced. Storage temperature was an important factor on the stability of crab apple anthocyanins. Anthocyanin loss in strawberry marmalades was observed at most in samples stored at 35 ℃. When the analysis results obtained in this study were examined, the most suitable results were found in the samples stored at 9 ℃ at the selected storage temperatures. According to the results obtained in this study, it can be recommended to use crab apple (*Malus floribunda*) anthocyanins in food production as a new natural colorant source. Crab apple will add functional properties when added to food products with its antioxidant activity.

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Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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