

# STABILITY OF BLACK CARROT ANTHOCYANINS IN THE TURKISH DELIGHT (LOKUM) DURING STORAGE

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Accepted for Publication April 21, 2009

## ABSTRACT

*In this research, traditional Turkish delight (lokum) was colored with black carrot juice concentrate, and the variations in anthocyanin content, during storage at different temperatures (12, 20 and 30C) for 5-month periods, were observed by spectrophotometer and high-performance liquid chromatography. Analysis of kinetic data suggested a first-order reaction for the degradation of black carrot anthocyanins in Turkish delight. Degradation rates of anthocyanins of black carrot increased with increasing temperature. It was determined that the degradation rate of black carrot anthocyanins during the storage period at 12C increased faster than that of the other temperatures (20 and 30C). The k values for 12, 20 and 30C were found to be  $6.91 \times 10^{-3}$ ,  $4.21 \times 10^{-3}$  and  $9.21 \times 10^{-3}$ /day, respectively. Effects of pH on the thermal stability of black carrot anthocyanins were also determined. Results showed that the stability of anthocyanins decreased as the pH value increased. Increase in pH values correlated well with the decrease in anthocyanin content of the samples during storage. In all of the samples, redness (a\*) decreased during the storage at all temperatures; however, lightness (L\*) increased.*

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## PRACTICAL APPLICATIONS

Most of commercial anthocyanins used as a colorant in foods are obtained from fruits or vegetables such as: red grape, elderberry, blackcurrant, blackberry, raspberry, black chokeberry, red cabbage, black carrot, purple corn, red radish and purple sweet potato.

Turkish delight (lokum), one of the most popular traditional food products in Turkey, is a famous Turkish desert known all over the world. Lokum is produced by using sugar, water, starch, citric acid, aromatic compounds, dried fruits and natural colorant. The color of foods, which is one of the initial properties noticed in foods, is one of the most important quality parameters affecting consumers. Because of consumer anxiety over the safety of synthetic food colorants, the demand for natural food colorants has increased. Particularly, there is an increasing request for natural red food colorants as alternatives to the most commonly used synthetic red colorant. Therefore, the availability and the suitability of black carrot juice concentrate as a natural colorant instead of synthetic colorants which are considered to have some negative properties for human health was investigated by this work.

## INTRODUCTION

Turkish delight is a traditional famous Turkish desert known all over the world. Turkish delight is produced by using sugar, water, starch, citric acid, aromatic compounds, dried fruits and natural colorant (Artık 1997; Artık *et al.* 2008). The color of foods, which is one of the initial properties noticed in foods, is one of the most important quality parameters affecting consumers (Kırca 2004). The demand for natural food colorants has increased due to consumer anxiety over the safety of synthetic food colorants. Particularly, there is an increasing request for natural red food colorants as alternatives to the most commonly used synthetic red colorant, FD&C Red #40 (Giusti and Wrolstad 2003). Natural red colorants such as betanin, cochineal (carmine and carminic acid), carotenoids (paprika, canthaxanthin) and anthocyanins are successfully used in foods (Askar 1993; Francis 1994). Especially, anthocyanins, well-known natural red colorants, are used in foods (Bridle and Timberlake 1997). In addition, concern over anthocyanins has increased due to their possible role in reducing the risk of several diseases such as coronary heart disease, cancer and stroke (Wrolstad 2004).

Most of commercial anthocyanins used as a colorant in foods are obtained from fruits or vegetables such as: red grape, elderberry, blackcurrant, blackberry, raspberry, black chokeberry, red cabbage, black carrot, purple corn, red radish and purple sweet potato. Among these, the largest natural

source for anthocyanins is red grape skin, followed by elderberry, black carrot and red cabbage (Downham and Collins 2000; Kırca *et al.* 2006). Four anthocyanin colorants derived from grape skin extract, grape color extract, fruit juice and vegetable juice are exempt from certification in the U.S.A. (Wrolstad 2004). The main problem with the application of anthocyanin colorants is related to their low stability to heat, light and pH changes (Kırca *et al.* 2006).

Stintzing *et al.* (2002) have reported that acylated anthocyanins are extremely resistant to hydration. So, they have high color stability at food pH. In fact, Stintzing *et al.* (2002) revealed that acylation with cinnamic acid considerably increased the stability of cyanidins isolated from black carrot, elderberry, blackberry, red cabbage and sweet potato colorants. However, glycosidic substitution at the 5 position lowered the stability. The major natural sources based on anthocyanin colorants including acylated anthocyanins are red radishes (Giusti and Wrolstad 1996), red potatoes (Rodriguez-Saona *et al.* 1999), red cabbages (Dyrby *et al.* 2001) and black carrots (Kırca *et al.* 2006).

The anthocyanin content of black carrots, which are a good source of anthocyanin pigments, was reported to be 439 mg/L juice (Kırca *et al.* 2007) and 1,750 mg/kg fresh weight (Mazza and Miniati 1993). In addition, black carrots also include high amounts of acylated anthocyanins. Stintzing *et al.* (2002) identified four major anthocyanins in black carrot extract and detected 41% of anthocyanins to be acylated, that is, cyanidin 3-sinapoyl-xylosyl-glucosyl-galactoside (27.5%) and cyanidin 3-feruloyl-xylosyl-glucosyl-galactoside (13.5%).

The purpose of the present work was to investigate the availability and the suitability of black carrot juice concentrate as a natural colorant instead of synthetic colorants which are considered to have some negative properties for human health, to follow any changes during storage and to evaluate its potential for Turkish delight product.

## MATERIALS AND METHODS

### Materials

In this study, black carrot juice concentrate is used as a source of pigment for giving red color to Turkish delight. Black carrot concentrate samples were obtained from TARGIT Fruit Juice Firm, Mersin. They were kept at  $-30^{\circ}\text{C}$  until use for the purpose of coloring the Turkish delights samples.

Turkish delight used in this study was produced in the plant of AZIM Confectionery Company, Konya. The flow diagram of Turkish delight is shown in Fig. 1. Also, 6.6 g of black carrot concentrate for per kilogram Turkish delight sample was used for the purpose of giving color to the Turkish delight.

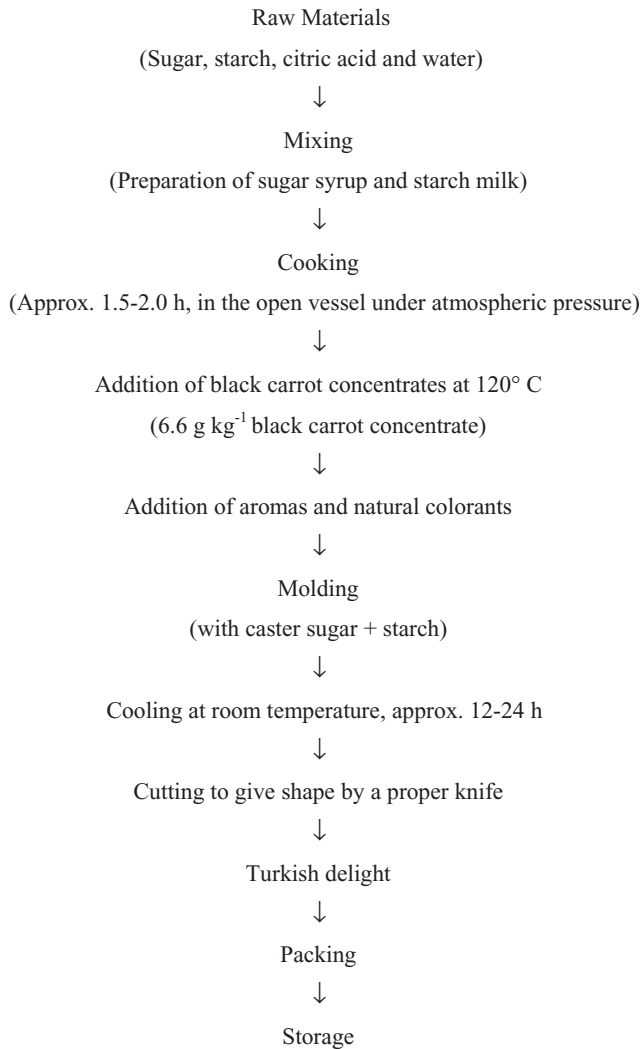


FIG. 1. PRODUCTION FLOW DIAGRAM OF TURKISH DELIGHT

### Turkish Delight Preparation and Storage

Crystal sugar and adequate water to dissolve it were poured into the open boiling vessel. Citric acid was dissolved in the other vessel. Starch used in Turkish delight was suspended in the remainder of the water. Then, the starch suspension and citric acid solution were added to the sugar solution and mixed.

After the mixture was heated to 120C, black carrot concentrate was added (Fig. 1).

When Turkish delight mass colored by black carrot juice concentrate reached desired thickness, the boiling was stopped, and then was poured onto the rectangle wooden tray. After this, the mass was rested for a period of about 12 to 24 h in the wooden tray for gelation of the product. Caster sugar and starch mix were sprinkled onto the mass in the wooden tray, and it was packed in 100-g portions by cutting it into small pieces with a cutter (Fig. 1).

At the end of packing, Turkish delight was divided into three portions. For storage, these samples were placed in a box and transferred into the temperature-controlled incubators at 12 and 20C  $\pm$  0.5C (Sanyo MIR 153, Gunma, Japan) and at 30C  $\pm$  0.1C (Mettler BE 400, Schwabach, Germany). At regular time intervals, 100 g Turkish delight samples were removed from the incubators and were first subjected to color analyses ( $L^*$ ,  $a^*$ ,  $b^*$ ) by cutting in half and then were analyzed for total anthocyanin content spectrophotometrically and by high-performance liquid chromatography (HPLC).

## Methods

**Color Analysis of the Turkish Delight During Storage.** Color measurements of the Turkish delight samples were performed by using a reflectance spectrophotometer (Minolta CM-3600d, Osaka, Japan) (Akbulut and Çoklar 2008).  $L^*$ ,  $a^*$  and  $b^*$  values of Turkish delight were measured by using Commission Internationale de l'Eclairage (CIE) LAB system. Then, chroma ( $C^*$ ) and hue angle ( $h^\circ$ ) were calculated from CIE  $a^*$  and  $b^*$  by means of Eqs. (1) and (2), respectively. All determinations were performed in duplicate.

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (1)$$

$$h^\circ = \arctan (b^*/a^*) \quad (2)$$

**Monomeric Anthocyanin Analysis.** Monomeric anthocyanin content of Turkish delight samples was determined by using the pH differential method (Giusti and Wrolstad 2003). An ultraviolet-visible (UV-VIS) double beam spectrophotometer (ThermoSpectronic Helios-a, Cambridge, England) was used for spectral measurements at 526 ( $k_{\max}$ ) and 700 nm. Pigment content was calculated based on the cyanidine-3-glucoside with a molecular weight of 445.2 g/L and a molar absorbance ( $\epsilon$ ) of 29,600 cm/mg.

In addition, anthocyanin composition and content of Turkish delight samples was determined by HPLC (Fig. 2). Results from HPLC and pH

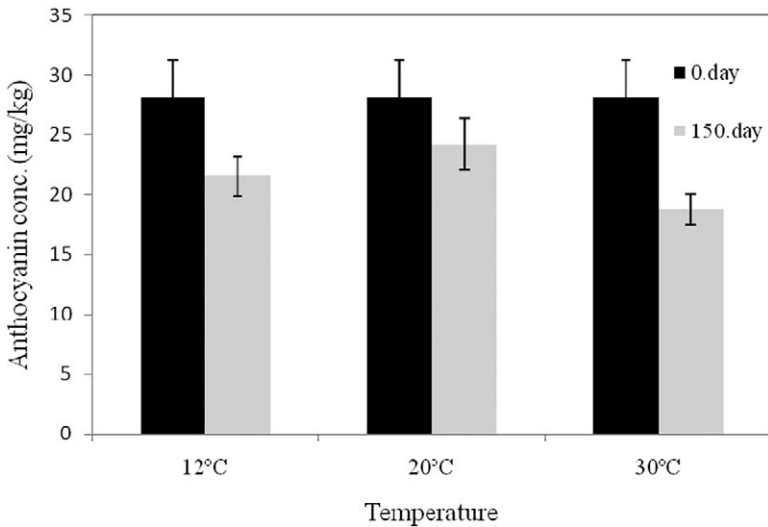


FIG. 2. CHANGES IN THE ANTHOCYANIN CONCENTRATE OBTAINED BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF TURKISH DELIGHT COLORED BY BLACK CARROT JUICE CONCENTRATE DURING STORAGE AT DIFFERENT TEMPERATURES

differential methods were compared with each other. Anthocyanins analyses by HPLC consist of three stages, including extraction, purification and definition and calculation.

**Extraction of Anthocyanins.** Turkish delight (100 g) was first homogenized with 100 mL distilled water on a Waring blender (Waring Commercial Laboratory Blender-Waring Products Division, Torrington, CT) for 30 min and 100 mL solvent (85% ethanol + 15% 1.5 N HCl). Then, 50 g of this homogenate was again homogenized by a homogenizer with a high cycle at 15,500 rpm (Heidolph SilentCrusher M, Schwabach, Germany) for 20 min.

The homogenized blend was filtered through Whatman No. 1 filter paper under a vacuum (Whatman International Ltd., Maidstone, England). After filtering, it was added into a separation funnel, including 200 mL chloroform. The separation funnel was shaken by moving back and forth quickly, and then, in order to create the phase separation, it was held in the refrigerator overnight at 4°C in darkness.

After removing the chloroform phase, the upper colored part containing the anthocyanins was evaporated by a rotary evaporator at 40°C under vacuum, in order to remove solvent and chloroform residue from the anthocyanin rich phase. The remaining watery extract was made up to 50 mL with the water

including 0.01% HCl (37.7% v/v) and filtered through polyvinylidene difluoride (PVDF) filter with 0.45  $\mu\text{m}$  pore diameter (Millipore, Bedford, MA) (Cemeroğlu 2007).

**Purification of Anthocyanins.** In order to remove the components such as sugar and organic acids from the extract, they were subjected to purification process. At the first stage of the purification process, C-18 Oasis cartridges (Waters, Milford, MA) were conditioned with 5 mL 0.01% HCl in methanol, and then 2 mL 0.01% HCl in distilled water; 50 mL extract and 15 mL distilled water were passed through them, respectively. While organic acids, sugars and other components dissolved in the water were removed from the extracts, anthocyanins and other polyphenolic compounds were bound to the filling material of the cartridge.

C-18 Oasis cartridges were dried by using nitrogen for 5 min. After dried cartridges were washed with methanol containing 0.01% HCl, extracts including anthocyanins were obtained. The anthocyanin extracts were dried by using nitrogen in a water bath at 40°C to remove methanol acidulated with 0.01% HCl.

After this stage, the dried anthocyanin extract in the tube was redissolved by adding 5 mL distilled water containing 0.01% HCl. This solution including anthocyanins was injected to HPLC by filtering through PVDF filter with 0.45  $\mu\text{m}$  pore diameter (Millipore, Bedford, MA)

The equipment consisted of a quaternary solvent delivery system (Agilent 1200 model, Waldbronn, Germany), online degasser, Rheodyn injection valve (Rheodyn, Cotati, CA) with a 50- $\mu\text{L}$  loop and an external oven coupled to a diode array detector. The acquisition and data processing were performed by the ChemStation rev. B.02.01 software (Agilent, Waldbronn, Germany). A 50- $\mu\text{L}$  sample was analyzed on a C-18 reversed-phase column (250  $\times$  4.6 mm i.d.; 5  $\mu\text{m}$  particle size) (Phenomenex Gemini, Los Angeles, CA) using as mobile phase acetonitrile (100%, v/v) (solvent A) and *o*-phosphoric acid/acetic acid/acetonitrile/water (1:10:5:84; v/v/v/v) (solvent B). Elution was performed at a flow rate of 1 mL/min using a gradient starting with 99B : 1A, v/v, increasing to 88B : 12A, v/v at 10 min, 78B : 22A, v/v at 25 min, and 99B : 1A, v/v at 30 min. The chromatograms were processed at 520 nm (Fig. 3) and the spectra were obtained between 250 and 600 nm. All analyses were done in duplicate and the results were expressed as mean values.

**Other Analyses.** pH, titratable acidity, soluble solids ( $^{\circ}\text{Brix}$ ), reducing sugar, total sugar, sucrose and ascorbic acid were determined according to Cemeroğlu (2007). To establish pH value of Turkish delight, 10 g of sample was firstly homogenized by a blender with 90 mL distilled water, and pH of the homogenate was determined by pH meter at 20°C (WTW Inolab Level 1, Weilheim, Germany); titratable acidity, expressed as percentage of citric acid,

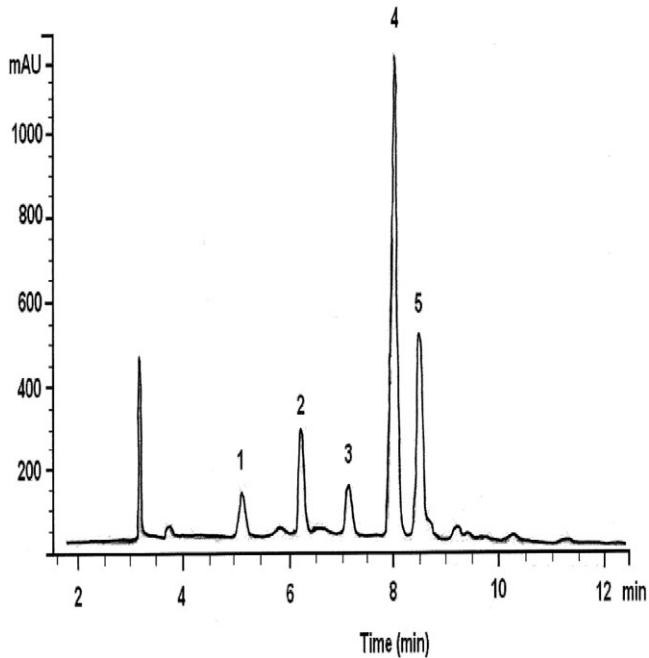


FIG. 3. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY CHROMATOGRAM OF ANTHOCYANIN EXTRACTED FROM TURKISH DELIGHT COLORED BY BLACK CARROT CONCENTRATE (PIGMENT CONTENT WAS CALCULATED ACCORDING TO AREAS OF 1, 2, 3, 4 AND 5 ANTHOCYANIDIN PIKES COMPARED WITH THAT OF CYANIDINE-3-GLUCOSIDE PIKE AND WAS EXPRESSED TO BE mg/kg)

was determined with 0.1 N NaOH up to pH 8.1; soluble dry matter was determined with an ATAGO model refractometer (Atago Co. Ltd., Tokyo, Japan). Total sugar, reducing sugar and sucrose were quantitated by the Lane-Eynon method (Cemeroğlu 2007).

## RESULTS AND DISCUSSION

Some physical and chemical characteristics of black carrot juice concentrate are shown in Table 1. The soluble solid portion of the black carrot concentrate was determined to be 60.4%. Monomeric anthocyanin content was 242.4 mg/100 mL in black carrot juice concentrate. Kammerer *et al.* (2004) determined that total anthocyanin contents of black carrots ranged from 45.4 mg/kg to 17.4 g/kg dry matter. Unexpectedly, black carrot concentrate included quite high amount of ascorbic acid (148.6 mg/100 mL).



TABLE 1.  
SOME PHYSICOCHEMICAL DATA OF BLACK CARROT  
JUICE CONCENTRATE USED IN TURKISH DELIGHT

|   |               |
|---|---------------|
| °Brix                                     | 60.4 ± 1.2    |
| pH value                                  | 5.6 ± 0.2     |
| Titrateable acidity* (g/100 mL)           | 0.642 ± 0.037 |
| Reducing sugar (g/100 mL)                 | 8.21 ± 0.98   |
| Total sugar (g/100 mL)                    | 24.8 ± 1.1    |
| Sucrose (g/100 mL)                        | 16.6 ± 1.3    |
| Ascorbic acid (mg/100 mL)                 | 148.6 ± 13.5  |
| Monomeric anthocyanin content (mg/100 mL) | 242.4 ± 12.56 |

\* As anhydrous citric acid.

TABLE 2.  
CHANGES IN THE ANTHOCYANIN CONTENT OF TURKISH DELIGHT COLORED  
WITH BLACK CARROT JUICE CONCENTRATE DURING STORAGE AT THE  
DIFFERENT TEMPERATURES\*

| Temperature<br>(C) | Anthocyanin content (mg/100 g) |                          |                          |                          |                          |
|--------------------|--------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
|                    | Storage periods (day)          |                          |                          |                          |                          |
|                    | 0                              | 60                       | 90                       | 120                      | 150                      |
| 12                 | 12.62 ± 0.55 <sup>ns</sup>     | 8.37 ± 0.04 <sup>b</sup> | 7.35 ± 0.02 <sup>b</sup> | 5.46 ± 0.01 <sup>b</sup> | 3.69 ± 0.29 <sup>b</sup> |
| 20                 | 12.62 ± 0.55 <sup>ns</sup>     | 8.77 ± 0.01 <sup>a</sup> | 8.32 ± 0.05 <sup>a</sup> | 6.34 ± 0.02 <sup>a</sup> | 5.27 ± 0.40 <sup>a</sup> |
| 30                 | 12.62 ± 0.55 <sup>ns</sup>     | 8.05 ± 0.23 <sup>c</sup> | 6.65 ± 0.12 <sup>c</sup> | 4.55 ± 0.17 <sup>c</sup> | 3.05 ± 0.12 <sup>b</sup> |

\* Means marked by different letters in the same columns are significantly different ( $P < 0.01$ ).  
<sup>ns</sup>, not significant.

### Changes in Anthocyanin Content during Storage

Changes in anthocyanin content during storage, according to the results obtained by using both pH differential and HPLC methods, are presented in Table 2 and Fig. 2, respectively. Initial anthocyanin content of the Turkish delight was 12.62 mg/kg. Anthocyanin content decreased in the course of time during the storage. The degradation of anthocyanins in Turkish delight at 12 to 30C fitted to a first-order kinetic model (Fig. 4), confirming the results of previous studies reporting the same model during storage (Sondheimer and Kertesz 1953; Skrede *et al.* 1992; Garzon and Wrolstad 2002; Kirca *et al.* 2007). The first-order reaction rate constants ( $k$ ) and half-life periods ( $t_{1/2}$ ), i.e., the time needed for 50% degradation of anthocyanins, were calculated by the following equations:

$$C_t/C_0 = \exp(-kt) \quad (3)$$

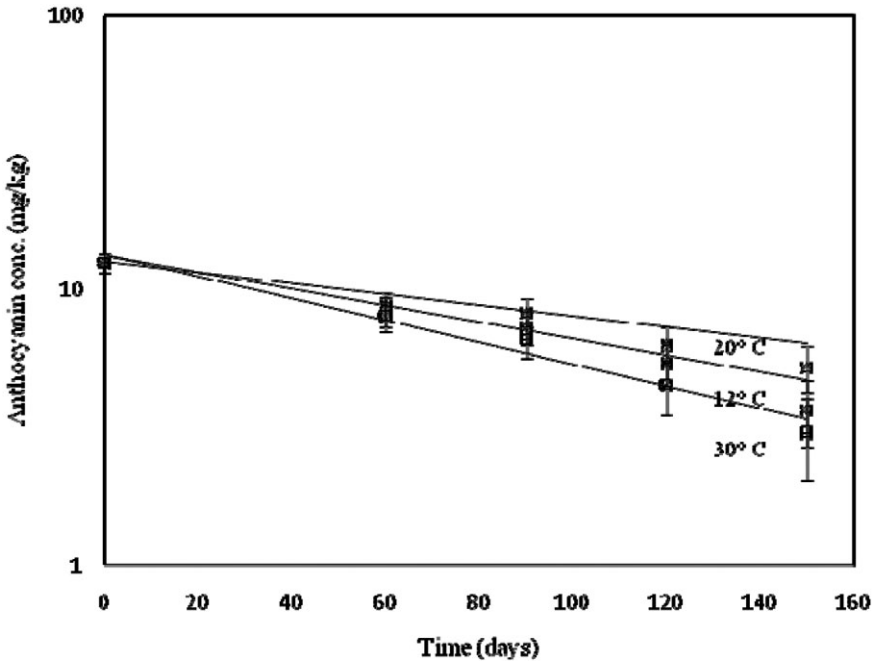


FIG. 4. DEGRADATION OF ANTHOCYANINS IN TURKISH DELIGHT COLORED BY BLACK CARROT JUICE CONCENTRATES DURING STORAGE AT DIFFERENT TEMPERATURES

$$t_{1/2} = -\ln(0.5) \times k^{-1} \quad (4)$$

In the equation above,  $C_0$  is the initial anthocyanin content (mg/kg) and  $C_t$  is the anthocyanin content (mg/kg) at  $t$  time storage (days). Storage time had significant effect on anthocyanin content at all three storage temperatures for Turkish delight ( $P < 0.01$ ).

As shown in the Table 2 and Fig. 1, storage temperature was the most effectual parameter on the anthocyanin degradation.

According to obtained values, anthocyanin degradation continued more quickly at lower and higher temperatures, but anthocyanin degradation at 20C was determined to be less than that at 12 and 30C (Fig. 1).

The anthocyanins of Turkish delight exhibited low stability during storage, as indicated by much lower  $t_{1/2}$  (Table 3). The  $t_{1/2}$  values of anthocyanins at 30C were 10.7, and as expected, storage at 30C resulted in a much faster degradation of anthocyanins. The  $t_{1/2}$  value of anthocyanins at 12C was lower than that of 20C. This was an unexpected state because 12C was the

TABLE 3.  
KINETIC PARAMETERS FOR THE DEGRADATION OF BLACK CARROT ANTHOCYANIN  
DURING STORAGE IN THE TURKISH DELIGHT

| Temperature<br>(C) | $-k \times 10^{-3}/\text{day}$ | $t_{1/2}$ (week) | $Q_{10}$ |        |
|--------------------|--------------------------------|------------------|----------|--------|
|                    |                                |                  | 12–20C   | 20–30C |
| 12                 | 6.91 (0.965)*                  | 14.3             | 0.61     | 2.00   |
| 20                 | 4.61 (0.978)                   | 21.4             |          |        |
| 30                 | 9.21 (0.972)                   | 10.7             |          |        |

\* Numbers in parentheses are the determination coefficients.

lowest temperature used in the study. As indicated by Kirca *et al.* (2007), half-life period for anthocyanins decrease with the increasing of temperature.

The effect of temperature on the degradation of black carrot anthocyanins was determined by calculating the temperature quotient ( $Q_{10}$ ) values from the following equations:

$$Q_{10} = (k_2/k_1)^{10/(T_2-T_1)} \quad (5)$$

While  $Q_{10}$  value between 20 and 30C was calculated to be 2.0, it was found to be 0.61 between 12 and 20C (Table 3). A very fast decrease in anthocyanin content was observed during storage at 12C; however, in this study, this was an unexpected case, too.

The cause of the fast degradation occurring at the lower temperature can be the retrogradation of starch in the Turkish delight composition. BeMiller and Whistler (1996) have reported that these retrogradation phenomena have a great importance in the staling of starch-rich products such as bread and others. The rate of staling is dependent on the product formulation and storage conditions. Staling is due, at least in part, to the gradual transition of amorphous starch to a partially crystalline, retrograded state. In starch-rich foods, where there is just enough moisture to gelatinize starch granules (while retaining a granule identity), amylose retrogradation, namely insolubilization may be largely completed by the time when the product has cooled to room temperature. Retrogradation of amylopectin is believed to involve primarily association of its outer branches and requires a much longer time than amylose retrogradation, giving it prominence in the staling process that occurs with time after the product has cooled (BeMiller and Whistler 1996). During this retrogradation process, water in the structure of starch of Turkish delight was released; therefore, free water in the products should be increased. It is well known that anthocyanins are compounds

TABLE 4.  
CHANGES IN THE WEIGHT (g) OF TURKISH DELIGHT  
COLORED WITH BLACK CARROT JUICE CONCENTRATE  
DURING STORAGE AT THE DIFFERENT TEMPERATURES

| Storage<br>time (day) | Sample weight (g) |       |       |
|-----------------------|-------------------|-------|-------|
|                       | 12C               | 20C   | 30C   |
| 0                     | 43.45             | 68.64 | 62.13 |
| 60                    | 43.47             | 67.62 | 58.54 |
| 90                    | 43.78             | 66.76 | 57.78 |
| 120                   | 44.34             | 65.99 | 57.11 |
| 150                   | 44.62             | 65.59 | 56.66 |

highly soluble in water. Thus, the reason for the rapid degradation of anthocyanin in Turkish delight samples stored at 12C may be the increase in the free water.

Water content data obtained from the Turkish delight samples supported this case (Table 4). While moisture content of Turkish delight during storage at 20 and 30C decreased, it increased at 12C. These results indicated that free water level increased in the Turkish delight colored with black carrot concentrate during the storage at the lower temperature.

### Changes in Color during Storage

In the Turkish delight, as well as anthocyanin loss, the stability of black carrot anthocyanins was also investigated by color measurement. For this aim, the CIE  $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*$  and  $h^\circ$  color values were used to observe the color changes in Turkish delight during storage. These color measurement values show parallelism with color concept perceived by human eyes.

The perceptible color of Turkish delight depends on the relative amount of red and yellow color, which is expressed as an angle of hue (Rein and Heinonen 2004). Hue angle ( $h^\circ$ ) describes the color as  $0^\circ/360^\circ$  for red-purple,  $90^\circ$  for yellow,  $180^\circ$  for green and  $270^\circ$  for blue (Kirca *et al.* 2007).

Results related to color measurements are given in Table 5. According to values in Table 5, while  $L^*$  value (lightness), which is the indication of browning index, increased during storage at all temperatures,  $a^*$  value, that is, redness, decreased ( $P < 0.05$ ). Aguilera *et al.* (1987) have reported that  $L^*$  value is used as a browning index in foods. It is known that there is a relationship between instrumental color measurement values performed with a reflectance colorimeter and several quality characteristics of foods, especially color deteriorations (Little 1976).

TABLE 5.  
COLOR CHANGES OF THE TURKISH DELIGHT SAMPLES DURING STORAGE AT THE DIFFERENT TEMPERATURE

| Time (day) | Color parameters† |           |              |            |               |
|------------|-------------------|-----------|--------------|------------|---------------|
|            | $L^*$             | $a^*$     | $b^*$        | $C^*$      | $h^\circ$     |
| At 12C     |                   |           |              |            |               |
| 0          | 26.02 ± 0.81      | 6.4 ± 0.6 | -0.28 ± 0.04 | 6.4 ± 0.61 | 357.46 ± 0.12 |
| 150        | 26.19 ± 1.71      | 5.3 ± 0.3 | -0.94 ± 0.12 | 5.4 ± 0.32 | 349.96 ± 0.17 |
| At 20C     |                   |           |              |            |               |
| 0          | 26.02 ± 0.81      | 6.4 ± 0.6 | -0.28 ± 0.04 | 6.4 ± 0.61 | 357.46 ± 0.12 |
| 150        | 26.53 ± 2.21      | 5.8 ± 0.8 | -1.20 ± 0.20 | 5.9 ± 0.83 | 348.39 ± 0.33 |
| At 30C     |                   |           |              |            |               |
| 0          | 26.02 ± 0.81      | 6.4 ± 0.6 | -0.28 ± 0.04 | 6.4 ± 0.61 | 357.46 ± 0.12 |
| 150        | 27.13 ± 1.35      | 4.8 ± 0.6 | -0.72 ± 0.04 | 4.8 ± 0.60 | 350.15 ± 0.59 |

† All values are expressed in mean ± standard deviation ( $n = 3$ ).

TABLE 6.  
CHANGES IN THE pH OF TURKISH DELIGHT COLORED WITH BLACK CARROT JUICE CONCENTRATE DURING STORAGE AT THE DIFFERENT TEMPERATURES

| Storage time (day) | Temperature           |                       |                       |
|--------------------|-----------------------|-----------------------|-----------------------|
|                    | 12C                   | 20C                   | 30C                   |
| 0                  | 3.99 <sub>22.4C</sub> | 3.99 <sub>22.4C</sub> | 3.99 <sub>22.4C</sub> |
| 60                 | 4.08 <sub>20.7C</sub> | 4.02 <sub>21.6C</sub> | 4.12 <sub>22.6C</sub> |
| 90                 | 4.15 <sub>20.5C</sub> | 4.08 <sub>21.7C</sub> | 4.28 <sub>22.7C</sub> |
| 120                | 4.27 <sub>20.1C</sub> | 4.12 <sub>21.3C</sub> | 4.35 <sub>22.9C</sub> |
| 150                | 4.39 <sub>20.9C</sub> | 4.14 <sub>21.9C</sub> | 4.58 <sub>23.0C</sub> |

### Changes in pH during Storage

In Table 6, the pH changes in the Turkish delight samples during storage at several temperatures were shown. As indicated in Table 6, pH values of Turkish delight samples increased during storage at all temperatures, especially at 30C ( $P < 0.05$ ). Also, while pH values increased, anthocyanin concentration in Turkish delight decreased in the course of storage period at all temperatures, that is, there was a strong reverse relationship between both (Table 7). The other study examined the relationship between pH and anthocyanin deterioration and supported this case (Kirca 2004).

These results found that since products occurring from the anthocyanin degradation probably had a buffering character, they raised pH values

TABLE 7.  
CORRELATION BETWEEN pH VALUE AND ANTHOCYANIN  
CONTENT CHANGES DURING STORAGE AT THE  
DIFFERENT TEMPERATURES

| Temperature<br>(C) | <i>r</i> | <i>P</i> value |
|--------------------|----------|----------------|
| 12                 | -0.959   | 0.010          |
| 20                 | -0.939   | 0.018          |
| 30                 | -0.949   | 0.014          |

(Table 6). As result of this, lightness ( $L^*$ ) increased and redness ( $a^*$ ) decreased. It has been well documented that pH has a strong influence on the stability of anthocyanins (Daravingas and Cain 1968; Tanchev and Joncheva 1973; Torskangerpoll and Andersen 2005). Actually, Kirca *et al.* (2007) reported that color stability of anthocyanins depended strongly on pH and anthocyanin structure.

## CONCLUSION

Findings obtained from this work clearly displayed that anthocyanins from black carrot juice had a good stability in the colored Turkish delight during storage. Thus, black carrot juice concentrate would be a good alternative for coloring Turkish delight. Storage temperature was an important factor on the stability of anthocyanins.

In this study, retrogradation was considered as the reason of anthocyanin degradation at 12C occurs because of storage in the cold environment. Therefore, Turkish delight colored by black carrot anthocyanins is not suitable to store at 12C or lower. At 20C, the color of Turkish delight samples colored with black carrot juice concentrate was still red after 150 days of storage, as indicated by low anthocyanin degradation rates. Therefore, Turkish delight should be stored at 20C for longer shelf life, but not at refrigeration temperatures. This work obviously displayed the potential use of black carrot concentrate as a natural colorant in Turkish delight in synthetic colorants stead.

## ACKNOWLEDGMENTS

This study is in part of Mr. Özen's MS Thesis and was supported by "Selcuk University Research Funds," Turkey (Grant No. 2007-08201013). The authors wish to thank Assoc. Professor Mehmet Özkan for the help in the

chemical analyses in his own laboratory. The authors also thank the Targid Fruit Juice Company for providing black carrot juice concentrate.

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