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Effects of temperature, solid content and pH on the stability of black carrot anthocyanins

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Abstract

Anthocyanin stability of black carrots was studied at various solid contents (11, 30, 45 and 64° Brix) and pHs (4.3 and 6.0) during both heating, at 70–90 °C, and storage at 4–37 °C. Monomeric anthocyanin degradation fitted a first-order reaction model. Degradation of monomeric anthocyanins increased with increasing solid content during heating, while it decreased during storage. For example, at pH 4.3, half-life periods for anthocyanins at 30, 45 and 64 \textdegree Brix were, respectively, 8.4, 6.9 and 5.2 h during heating at 80 \textdegree C and 18.7, 30.8 and 35.9 weeks during storage at 20 °C. At 30–64° Brix, increasing pH from 4.3 to 6.0 enhanced the degradation of anthocyanins during heating. The effect of pH on thermal stability of anthocyanins was also studied at six different pHs (2.5–7.0) in citrate-phosphate buffer solutions and significant decrease in anthocyanin stability was observed at pHs above 5.0. Higher activation energies (E_a) were obtained during heating than during storage with increasing solid contents. At 30–64° Brix, E_a values ranged from 68.8 to 95.1 kJ mol⁻¹ during heating and from 62.1 to 86.2 kJ mol⁻¹ during storage. Q_{10} values at 20–37 °C were as high as 3.1 at 45° Brix and 3.6 at 64° Brix. 2006 Elsevier Ltd. All rights reserved.

Keywords: Black carrot; Anthocyanin; Stability

1. Introduction

Anthocyanins are well-known natural colorants, which provide bright red colour in foods ([Bridle & Timberlake,](#page-5-0) [1997\)](#page-5-0). In addition to colorant properties, interest in anthocyanins has intensified because of their possible role in reducing the risk of coronary heart disease, cancer and stroke ([Wrolstad, 2004\)](#page-6-0). Due to their high reactivity, anthocyanins readily degrade and form colourless or undesirable brown-coloured compounds. The primary problem with the application of anthocyanins as food colorants is their vulnerability to temperature (Cemeroğlu, Velioğlu, [& I](#page-5-0)şık, 1994; Kırca & Cemeroğlu, 2003), pH ([Tanchev &](#page-6-0) [Joncheva, 1973; Fossen, Cabrita, & Andersen, 1998; Ceval-](#page-6-0) [los-Casals & Cisneros-Zevallos, 2004](#page-6-0)), oxygen [\(Starr &](#page-6-0) [Francis, 1968](#page-6-0)), ascorbic acid [\(Shrikhande & Francis,](#page-6-0) [1974; Poei-Langston & Wrolstad, 1981\)](#page-6-0) and hydrogen peroxide (Sondheimer & Kertesz, 1952; Ozkan, Yemenicioğlu, Asefi, & Cemeroğlu, 2002).

Black carrot anthocyanins have higher colour stability at food pH than have anthocyanins from other plants. They especially provide an excellent bright strawberry-red shade at acidic pH's; therefore, black carrot juice can be a good choice for colouring fruit juices and nectars, soft drinks, conserves, jellies and confectionery ([Downham &](#page-5-0) [Collins, 2000\)](#page-5-0). The higher stability of anthocyanins from black carrot was attributed to the presence of acylated groups. [Cevallos-Casals and Cisneros-Zevallos \(2004\)](#page-5-0) showed that acylated anthocyanins were more stable to pH and temperature changes than were non-acylated ones. [Kammerer, Carle, and Schieber \(2004\)](#page-6-0) reported that the proportion of acylated anthocyanins in black carrots from 15 different cultivars ranged from 55% to 99%, in most

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cases exceeding 80%. Similarly, [Stintzing, Stintzing, Carle,](#page-6-0) [Frei, and Wrolstad \(2002\)](#page-6-0) identified four major anthocyanins in black carrot extract and found 41% of anthocyanins to be acylated, namely cyanidin 3-sinapoly-xylosyl-glucosyl-galactoside (27.5%) and cyanidin 3-feruloyl-xylosyl-glucosyl-galactoside (13.5%).

In addition to their high anthocyanin stability, black carrots are a good source of anthocyanin pigments. [Mazza](#page-6-0) [and Miniati \(1993\)](#page-6-0) reported the anthocyanin content of black carrots to be 1750 mg kg^{-1} fresh weight. Recently, [Kammerer et al. \(2004\)](#page-6-0) found that total anthocyanin contents of black carrots ranged from 45.4 mg kg^{-1} to 17.4 $g kg^{-1}$ dry matter. Moreover, black carrots possess high antioxidant activity [\(Kaur & Kapoor, 2002; Uyan,](#page-6-0) [Baysal, Yurdagel, & El, 2004\)](#page-6-0) and contain a high amount of nutraceutical components ([Alasalvar, Grigor, Zhang,](#page-5-0) [Quantick, & Shahidi, 2001](#page-5-0)). Therefore, colouring of foods with black carrot juice may provide a health benefit as well. Furthermore, black carrot juice does not require declaration with an E-number on food labels when added to foods as a colourant.

There is only one study found in the literature for the stability of black carrot anthocyanins [\(Turker, Aksay, &](#page-6-0) [Ekiz, 2004\)](#page-6-0). In this study, the effect of temperature on the stability of black carrot anthocyanins was studied in shalgam, i.e., a fermented drink prepared from black carrots, during storage at 4, 25 and 40 °C. However, as indicated, shalgam is a black carrot drink and contains only a small amount of black carrot juice. More importantly, it contains other ingredients, which may change the stability of black carrot anthocyanins. The objective of our study was to determine: (1) the stability of anthocyanins in both pure black carrot juice and concentrates during heating and storage at various temperatures, soluble solids and pHs, and (2) the stability of black carrot anthocyanins in citrate-phosphate buffer solutions at different pHs during heating.

2. Materials and methods

2.1. Materials

Black carrots (Daucus carota L. ssp. sativus var. atrorubens Alef.) were obtained from Targid Fruit Juice Company, Mersin. The average values of root diameters, length and weight for black carrots were 33.1 mm (middle part) and 24.4 mm (upper part), 233 mm and 111.5 g, respectively. The carrots were processed in the fruit juice pilot plant of the University's Department of Food Engineering. After washed in cold tap water, carrots were ground and pressed on a rack and cloth press (Bucher-Guyer, Niederweningen, Switzerland). The pH of the juice was adjusted from 6.0 to 4.3 with 20% citric acid and then the juice was depectinized with the enzyme Panzym P5 (Begerow, Langenlonsheim, Germany) at 50° C for 2 h. The depectinized juice was filtered and then concentrated to 30, 45 and 64° Brix by a rotary low-pressure evaporator. To study the effect of pH on the thermal stability of black carrot anthocyanins, half of the concentrate samples were brought to pH 6.0 with 20% NaOH.

2.2. Methods

2.2.1. Degradation studies

The thermal stability of anthocyanins from black carrots was studied at 70, 80 and 90 °C. The samples at 11, $30, 45$ and 64° Brix were divided into 10 ml portions and filled in to Pyrex tubes. The tubes were well capped to avoid evaporation and placed in a thermostatic water bath (Memmert WB 14, Schwabach, Germany) preheated to a given temperature. At regular time intervals, samples were removed from the water bath and rapidly cooled by plunging into an ice water bath. The contents of heated and cooled tubes were analyzed for monomeric anthocyanin content.

The effect of pH on thermal stability was also studied at six different pHs (2.5, 3.0, 4.0, 5.0, 6.0 and 7.0) at the same temperatures. Citrate-phosphate buffer solutions at these pHs were prepared and then coloured with black carrot juice concentrate (1.5 g concentrate/100 ml solution). The coloured solutions were used for heating studies without further treatment. Finally, storage stability was determined at 4, 20 and 37 °C. The concentrates at 30, 45 and 64 \degree Brix were divided into 10 ml portions and filled into glass bottles. The bottles were then hermetically capped and pasteurized in the same water bath at 85° C for 15 min to prevent microbial growth during the storage period. The pasteurized concentrates were transferred into the incubators at 4° C (Sanyo MIR 153, Gunma, Japan) and, at 20 and 37 °C (Memmert BE 400, Schwabach, Germany).

2.2.2. Monomeric anthocyanin content

Monomeric anthocyanin contents of samples were determined using the pH-differential method described by [Giusti and Wrolstad \(2001\)](#page-5-0). Aliquots of black carrot juice/concentrate were brought to pH 1.0 and 4.5 and allowed to equilibrate for 1 h. The absorbance of each equilibrated solution was then measured at 530 nm (λ_{max}) and 700 nm for haze correction, using a UV–VIS doublebeam spectrophotometer (ThermoSpectronic Helios-a, Cambridge, England). Pigment content was calculated, based on cyanidin-3-glucoside [\(Alasalvar, Al-Farsi, Quan](#page-5-0)[tick, Shahidi, & Wiktorowicz, 2005\)](#page-5-0) with molecular weight of 445.2 and extinction coefficient of 29,600 ([Giusti &](#page-5-0) [Wrolstad, 2001](#page-5-0)).

Visible spectra of samples were determined by scanning the absorbance between 350 and 700 nm. Quartz cuvettes of 1 cm path-length were used and all measurements were carried out at room temperature (\sim 22 °C). Absorbance readings were made against distilled water as a blank.

2.2.3. Other analyses

Brix was measured at 20 \degree C using a digital refractometer (Atago Rx-7000 α , Tokyo, Japan) and pH with a pH meter (WTW Inolab Level 1, Weilheim, Germany). Titratable

acidity was determined according to the method outlined by [IFJU \(1968\)](#page-6-0) and expressed as "g citric acid 100 ml^{-1} juice''. Sugar contents were determined using the Lane-Eynon method and expressed as " $g l^{-1}$ " ([Anonymous,](#page-5-0) [1970\)](#page-5-0). Ascorbic acid content was determined using the 2.6-dichlorophenolindophenol-xylene extraction method and expressed as "mg 100 ml^{-1} juice" ([Anonymous, 1951\)](#page-5-0).

3. Results and discussion

3.1. General

Physical and chemical characteristics of the non-clarified black carrot juice are presented in Table 1. After clarification, monomeric anthocyanin content was 439 mg l^{-1} in black carrot juice. Surprisingly, black carrot juice contained high amount of ascorbic acid (26.4 mg 100 ml^{-1}).

3.2. Stability of monomeric anthocyanins during heating

Thermal stability of anthocyanins from black carrots was studied at various solid contents $(11, 30, 45, 64^{\circ}$ Brix) and pHs $(4.3, 6.0)$ at 70, 80 and 90 °C. Monomeric anthocyanin degradation followed a first-order reaction model (Fig. 1). Our results are in agreement with those from the previous studies, reporting a first-order reaction model for the degradation of monomeric anthocyanins from various sources [\(Culpepper & Caldwell, 1927; Garzon &](#page-5-0) Wrolstad, 2002; Kırca & Cemeroğlu, 2003). The first-order reaction rate constants (k) and half-lives $(t_{1/2})$, i.e. the time needed for 50% degradation of anthocyanins, were calculated by the following equations:

$$
\ln(C_t/C_0) = -\mathbf{k} \times t,\tag{1}
$$

$$
t_{1/2} = -\ln 0.5 \times k^{-1},\tag{2}
$$

where C_0 is the initial monomeric anthocyanin content and C_t is the monomeric anthocyanin content after t minute heating at a given temperature.

The degradation rate of anthocyanins increased with increasing heating temperature and solid content (Table 2). At 70–90 °C and pH 4.3, the $t_{1/2}$ values of black carrot anthocyanins ranged from 16.7 to 5.0 h at 11° Brix, 17.0 to 4.5 h at 30° Brix, 14.8 to 3.2 at 45° Brix and 14.4 to 2.3 at 64° Brix. Since no kinetic data have been found in the literature on the thermal degradation of black carrot antho-

Fig. 1. Degradation of anthocyanins in black carrot juice concentrate at 45° Brix and pH 4.3 during heating.

Table 2

Kinetic parameters for the thermal degradation of black carrot anthocyanins

Brix	Temperature $(^{\circ}C)$	$-k \times 10^3$ (min ⁻¹)	$t_{1/2}$ (h)
pH 4.3			
11	70	$0.69(0.951)^a$	16.7
	80	1.15(0.983)	10.1
	90	2.30(0.980)	5.0
30	70	0.68(0.976)	17.0
	80	1.38(0.955)	8.4
	90	2.57(0.961)	4.5
45	70	0.78(0.961)	14.8
	80	1.68(0.991)	6.9
	90	3.57 (0.987)	3.2
64	70	0.80(0.995)	14.4
	80	2.22 (0.994)	5.2
	90	4.98 (0.995)	2.3
pH 6.0			
11	70	0.69(0.969)	16.7
	80	1.15(0.992)	10.1
	90	2.30 (0.987)	5.0
30	70	0.87(0.992)	13.3
	80	1.75(0.989)	6.6
	90	3.63 (0.972)	3.2
43	70	0.93(0.982)	12.4
	80	2.05(0.981)	5.6
	90	4.20(0.991)	2.8
58	70	0.98(0.952)	11.8
	80	2.62(0.989)	4.4
	90	4.98 (0.977)	2.3

^a Numbers in parentheses are the determination coefficients.

cyanins, we compared the thermal stability of black carrot anthocyanins with other anthocyanin sources. Cemeroğlu [et al. \(1994\)](#page-5-0) reported that the $t_{1/2}$ values of sour cherry

anthocyanins at 60, 70 and 80 $^{\circ}$ C were, respectively, 24.0, 10.9 and 4.4 h at 45° Brix; and 13.1, 5.9 and 2.8 h at 71° Brix. At similar temperatures and solid contents, [Kırca](#page-6-0) and Cemeroğlu (2003) found that the $t_{1/2}$ values of blood orange anthocyanins at 70, 80 and 90 $\mathrm{^{\circ}C}$ were, respectively, 3.4, 1.3 and 0.7 h at 45° Brix; and 2.0, 0.8 and 0.4 h at 69° Brix. At 45 $^{\circ}$ Brix and 70 $^{\circ}$ C, the $t_{1/2}$ values of anthocyanins from black carrot, sour cherry and blood orange were 14.8, 10.9 and 3.4 h, respectively. These results clearly indicate that anthocyanins from black carrot are the most heat-stable, followed by those from sour cherry and blood orange. Anthocyanins from black carrot and blood orange have been reported to contain acylated anthocyanins. [Giusti](#page-5-0) [and Wrolstad \(1996\)](#page-5-0) indicated that acylated anthocyanins show unusual stability in neutral or weakly acidic media. [Kammerer et al. \(2004\)](#page-6-0) showed that black carrots from Amasya, Konya and Afyon region of Turkey contained 55%, 87% and 93% of their anthocyanins as acylated forms, respectively. Since black carrots used in this study also came from the Konya region, we can assume that black carrot anthocyanins in our study should contain over 80% acylated anthocyanins. Moreover, [Maccarone, Mac](#page-6-0)[carone, and Rapisarda \(1985\)](#page-6-0) reported that at least 18% of the anthocyanins from blood orange were acylated. Recently, [Hillebrand, Schwarz, and Winterhalter \(2004\)](#page-5-0) found that 30% of the blood orange anthocyanins were acylated. To the best of our knowledge, the presence of acylation has not been shown for the anthocyanins from sour cherry. However, we suspect that sour cherry should contain acylated anthocyanins to a certain extent because its anthocyanins showed higher stability than those from blood orange. Higher stability of black carrot anthocyanins could, therefore, be attributed to the presence of much higher amounts of acylated anthocyanins.

The degradation rates of anthocyanins increased with increasing solid content during heating. This is because reacting molecules become closer when a product is concentrated ([Nielsen, Marcy, & Sadler, 1993](#page-6-0)). Similar trends were observed for sour cherry anthocyanins (Cemeroğlu [et al., 1994\)](#page-5-0) and blood orange anthocyanins [\(Kırca &](#page-6-0) Cemeroğlu, 2003). The effect of pH on the stability of black carrot anthocyanins was studied in juice and concentrates at pH 4.3 and 6.0 ([Table 2\)](#page-2-0). It has been well documented that pH has a strong influence on the stability of anthocyanins ([Daravingas & Cain, 1968; Tanchev & Joncheva,](#page-5-0) [1973; Fossen et al., 1998\)](#page-5-0). In fact, [Torskangerpoll and](#page-6-0) [Andersen \(2005\)](#page-6-0) reported that colour stability of anthocyanins depended highly on pH and anthocyanin structure. As the juice became concentrated, increasing the pH from 4.3 to 6.0 hastened the degradation of anthocyanins [\(Table](#page-2-0) [2\)](#page-2-0).

The effect of pH on the stability of black carrot anthocyanins was also studied in citrate phosphate buffer solutions at six different pHs (2.5, 3.0, 4.0, 5.0, 6.0 and 7.0). As seen in Table 3, black carrot anthocyanins showed two distinct stability profiles: (1) at lower pHs (pH 2.5, 3.0 and 4.0), and (2) at higher pHs (pH 5.0, 6.0 and 7.0).

Table 3

Kinetic parameters for the thermal degradation of black carrot anthocyanins in citrate-phosphate buffers

pН	Temperature $(^{\circ}C)$	$-k \times 10^3$ (min ⁻¹)	$t_{1/2}$ (h)	$E_{\rm a}$ (kJ mol ⁻¹)
2.5	70	$0.46 (0.931)^a$	25.1	78.1 (0.996)
	80	0.92(0.994)	12.6	
	90	2.07(0.975)	5.6	
3.0	70	0.46(0.984)	25.1	72.4 (0.862)
	80	1.15(0.978)	10.0	
	90	1.84(0.979)	6.3	
4.0	70	0.46(0.961)	25.1	72.4 (0.862)
	80	1.15(0.991)	10.0	
	90	1.84(0.986)	6.3	
5.0	70	0.69(0.989)	16.7	56.8 (0.997)
	80	1.15(0.995)	10.0	
	90	2.07(0.995)	5.6	
6.0	70	0.92(0.943)	12.6	42.0 (0.999)
	80	1.38(0.981)	8.4	
	90	2.07(0.989)	5.6	
7.0	70	0.92(0.961)	12.6	47.4 (0.993)
	80	1.38(0.990)	8.4	
	90	2.30 (0.998)	5.0	

^a Numbers in parentheses are the determination coefficients.

Significant decrease in anthocyanin stability was observed at pHs above 5.0, as indicated by low $t_{1/2}$ values (Table 3). Similarly, thermal stabilities of cyanidin 3-rutinoside and peonidin 3-rutinoside in citrate buffers [\(Tanchev &](#page-6-0) [Joncheva, 1973\)](#page-6-0) and cyanidin 3-diglucoside in model systems ([Daravingas & Cain, 1968\)](#page-5-0) were reported to decrease as the pH increased. In contrast, [Cevallos-Casals and Cisn](#page-5-0)[eros-Zevallos \(2004\)](#page-5-0) reported that anthocyanins from the extracts of red sweet potato, purple corn and commercial purple carrot colorant were more resistant to 98 \degree C at pH 3 than at pH 1.

3.3. Stability of anthocyanins during storage

The degradation of monomeric anthocyanins from black carrot was also studied in concentrates of 30, 45 and 64° Brix during storage at 4, 20 and 37 °C. The degradation was also fitted a to first-order reaction model ([Fig. 2\)](#page-4-0). Storage temperature had a strong influence on the degradation of anthocyanins. In fact, storage at 37° C resulted in a much faster anthocyanin degradation as compared to refrigerated storage at 4 °C. For example, the $t_{1/2}$ values of black carrot anthocyanins at 30° Brix were 71.8 weeks at 4° C and only 4.1 weeks at 37 $^{\circ}$ C ([Table 4](#page-4-0)).

The only study on the stability of black carrot anthocyanins was carried out by [Turker et al. \(2004\)](#page-6-0), who reported that the $t_{1/2}$ values of anthocyanins in shalgam drink were 34, 11 and 3 weeks at 4, 25 and 40 \degree C, respectively. However, shalgam drink is not a pure black carrot juice and also contains other ingredients, such as salt, turnip and red chili powder. These ingredients may increase or

Fig. 2. Degradation of anthocyanins in black carrot juice concentrate 45° Brix and pH 4.3 during storage.

Table 4 Kinetic parameters for the degradation of black carrot anthocyanins (pH 4.3) during storage

Brix	Temperature $(^{\circ}C)$	$-k \times 10^3$ (day ⁻¹)	$t_{1/2}$ (week)
30	4	$1.38~(0.985)^a$	71.8
	20	5.30 (0.974)	18.7
	37	24.4 (0.992)	4.1
45	4	1.15(0.986)	86.1
	20	3.22(0.972)	30.8
	37	22.1 (0.994)	4.5
64	4	0.46(0.934)	215
	20	2.76(0.959)	35.9
	37	24.89 (0.971)	4.0

Numbers in parentheses are the determination coefficients.

decrease the stability of anthocyanins. Therefore, the stability of anthocyanins from black carrot juice at various solid contents was compared with those from other juices.

The calculated $t_{1/2}$ values for black carrot anthocyanins at 4, 20 and 37 °C were, respectively, 71.8, 18.7 and 4.1 weeks at 30° Brix, 86.1, 30.8 and 4.5 weeks at 45 $^{\circ}$ Brix, and 215.3, 35.9 and 4.0 weeks at 64° Brix. At the same temperatures, the $t_{1/2}$ values of anthocyanins were reported to be between 2.1–55.7 and 3.1–115.7 days in blood orange juice concentrates of 45 and 69° Brix (Kırca & Cemeroğlu, [2003\)](#page-6-0), and 14–310 and 11–356 days in sour cherry juice concentrates of 45 and 71° Brix (Cemeroğlu et al., 1994), respectively. [Rodriguez-Saona, Giusti, and Wrolstad](#page-6-0) [\(1999\)](#page-6-0) reported that the $t_{1/2}$ values of anthocyanins from red-fleshed potato and red radish juice concentrates in juice model system were 10 and 16 weeks during storage at 25 °C, respectively. [Iversen \(1999\)](#page-6-0) found the $t_{1/2}$ value of blackcurrant anthocyanins to be 23.6 weeks at 20 $^{\circ}$ C. These observations clearly indicate that anthocyanins from black carrot have greater stability than those from blood orange, sour cherry, red-fleshed potato, red radish and blackcurrant during storage.

Although increasing solid content resulted in a higher degradation of anthocyanins during heating, the degradation of anthocyanins progressed at a slower rate with increasing solid content during storage. For example, the $t_{1/2}$ value at 4 °C was 71.8 weeks at 30° Brix and 215.3 weeks at 64° Brix. Similar trends were observed for sour cherry anthocyanins (Cemeroğlu et al., 1994) and blood orange anthocyanins [\(K](#page-6-0)ırca & Cemeroğlu, 2003). However, [Garzon and Wrolstad \(2002\)](#page-5-0) found that pelargonidin-based anthocyanins degraded much faster in strawberry juice concentrate $(65^{\circ}$ Brix) than in strawberry juice (8 \degree Brix) during storage at 25 \degree C.

3.4. Temperature dependence

The dependence of the degradation of black carrot anthocyanins on temperature was determined by calculating the activation energy (E_a) and temperature quotient (Q_{10}) values from the following equations:

$$
k = k_0 e^{-E_a/RT},\tag{3}
$$

$$
Q_{10} = (k_2/k_1)^{10/(T_2 - T_1)}.
$$
\n(4)

Increasing solid content resulted in higher E_a values during both heating at 70–90 °C and storage at 4–37 °C ([Table 5\)](#page-5-0). As compared to heating, lower E_a values were obtained for the degradation of black carrot anthocyanins during storage. At 30–64 \degree Brix, the E_a values ranged from 68.8 to 95.1 kJ mol⁻¹ during heating and 62.1 to 86.2 kJ mol⁻¹ during storage, respectively. Since high activation energy reactions are more sensitive to temperature changes, black carrot anthocyanins are more susceptible to temperature elevation during heating than during storage.

The calculated E_a values for the degradation of black carrot anthocyanins are similar to those reported by Cemeroğlu et al. (1994) for sour cherry juice concentrates of 45–71° Brix (75.9–80.1 kJ mol⁻¹) at 50–80 °C and by Kırca and Cemeroğlu (2003) for blood orange juice concentrates of $45-69^{\circ}$ Brix $(84.5-89.5 \text{ kJ mol}^{-1})$ at 70-90 °C. Much lower E_a values for the degradation of anthocyanins were reported by [Turker et al. \(2004\)](#page-6-0) for shalgam drink $(46.5 \text{ kJ mol}^{-1})$ at $4-40 \degree C$ and by [Dyrby, Westerg](#page-5-0)[aard, and Stapelfeldt \(2001\)](#page-5-0) for drink model systems of blackcurrant and elderberry (50 and 56 kJ mol⁻¹, respectively) at 25–80 °C. The lower E_a values could be attributed to the much lower solid contents of drink model systems and shalgam drink. And also, the composition of shalgam drink may have exerted a degradative effect during storage.

At $11-64^\circ$ Brix, there were no systematic differences among the E_a values for the thermal degradation of black carrot anthocyanins at pH 4.3 and 6.0. However, two distinct E_a profiles were obtained for black carrot anthocyanins in citrate-phosphate buffers: (1) at lower pHs (2.5, 3.0 and 4.0), and (2) at higher pHs (5.0, 6.0 and 7.0). The

Table 5 Effect of temperature on the degradation of black carrot anthocyanins

Brix	$E_{\rm a}^{a}$ (kJ mol ⁻¹)	Q_{10}		E_{a}^{b} (kJ mol ⁻¹)	Q_{10}	
		70–80 °C	80–90 \degree C		4–20 $\mathrm{^{\circ}C}$	$20-37$ °C
pH 4.3						
11	62.5°	1.7	2.0			
30	68.8	2.0	1.9	62.1	2.3	2.5
45	78.8	2.2	2.1	63.7	1.9	3.1
64	95.1	2.8	2.2	86.2	3.1	3.6
pH 6.0						
11	62.5	1.7	2.0			
30	74.5	2.0	2.1			
43	78.2	2.2	2.1	\sim		
58	84.5	2.7	1.9	$\overline{}$		

 $^{\rm a}$ At 70–90 °C. $^{\rm a}$ At 70–90 °C.
^b At 4–37 °C.

 \degree Determination coefficients for E_a values were over 0.98.

 E_a values at lower pHs were significantly higher than those at higher pHs. For example, the calculated E_a values ranged from 78.1 to 72.4 kJ mol⁻¹ at pH 2.5-4.0 and 56.8 to 47.4 kJ mol^{-1} at pH 5.0–7.0, respectively [\(Table 3\)](#page-3-0).

Almost same Q_{10} values at 11–64 \degree Brix were obtained for the degradation of black carrot anthocyanins at pH 4.3 and 6.0 during storage (Table 5). The Q_{10} values at 20–37 °C were as high as 3.1 at 45° Brix and 3.6 at 64° Brix. High Q_{10} values at 20–37 °C clearly indicate that low storage temperatures are needed to prevent the degradation of anthocyanins in black carrot juice concentrates.

4. Conclusion

Results from this study showed that stability of anthocyanins in black carrot juice and concentrates depended on temperature, solid content and pH. Increasing solid content, pH and temperature, during both heating and storage, increased the degradation rates of anthocyanins. To minimize anthocyanin degradation, we recommend that black carrot concentrates be cooled, possibly to refrigeration temperatures, as soon as produced. Compared to the stability of anthocyanins from other sources in the literature, anthocyanins from black carrot showed greater stability to heat and pH changes. Such high stability may be attributed to the diacylation of the anthocyanin structure.

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