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Stability of black carrot anthocyanins in various fruit juices and nectars

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Abstract

Fruit juices (apple, grape, orange, grapefruit, tangerine and lemon) and nectars (apricot, peach and pineapple) were coloured with black carrot juice concentrate and stability of black carrot anthocyanins in these matrices was studied during heating at 70–90 °C and storage at 4–37 °C. Anthocyanin degradation, in all coloured juices and nectars, followed first-order reaction kinetics. During heating, black carrot anthocyanins in apple and grape juices showed higher stability than those in citrus juices at 70 and 80 °C. High stability was also obtained for the anthocyanins in peach and apricot nectars at these temperatures. Black carrot anthocyanins were the least stable in orange juice during both heating and storage. During storage, degradation of anthocyanins was very fast at 37 °C, especially in pineapple nectar. Refrigerated storage (4 °C) markedly increased the stability in all samples. Activation energies for the degradation of black carrot anthocyanins in coloured juices and nectars ranged from 42.1 to 75.8 kJ mol^{-1} at 70–90 °C and 65.9–94.7 kJ mol^{-1} at 4–37 °C.

Keywords: Anthocyanins; Stability; Black carrot; Fruit juices; Nectars

1. Introduction

Consumer concern over the safety of synthetic food colorants has increased the demand for natural food colorants. Especially, there is a growing demand for natural red food colorants as alternatives to the most commonly used synthetic red colorant, FD&C Red #40 (Giusti & Wrolstad, 2003). Natural red colorants permitted in foods include betanin, cochineal (carmine and carminic acid), carotenoids (paprika, canthaxanthin) and anthocyanins (Askar, 1993; Francis, 1994). Among these, anthocyanins are the best-known natural red colorant used in foods (Bridle & Timberlake, 1997). 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).

Commercial anthocyanin colorants are mostly derived from fruits and vegetables. These sources include red grape, elderberry, blackcurrant, blackberry, raspberry, black chokeberry, red cabbage, black carrot, purple corn, red radish and purple sweet potato. The largest natural commercial source for anthocyanins is red grape skin, followed by elderberry, black carrot and red cabbage (Downham & Collins, 2000). In the USA, 4 anthocyanin-derived colorants, namely grape skin extract, grape colour extract, fruit juice and vegetable juice,

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are exempt from certification (Wrolstad, 2004). The primary problem with the application of anthocyanin colorants is the low stability to heat, light and pH changes. For example, at pH over 1, anthocyanins become hydrated at the 2 position and this leads to the formation of colourless hemiketals. It has been reported that acylated anthocyanins are more resistant to hydration; therefore, they possess high colour stability at food pH. In fact, Stintzing, Stintzing, Carle, Frei, and Wrolstad (2002) showed that acylation with cinnamic acid substantially increased the stability of cyanidins isolated from black carrot, red cabbage, sweet potato, elderberry and blackberry colorants, whereas glycosidic substitution at the 5 position lowered the stability. The main natural sources of anthocyanin-based colorants containing acylated anthocyanins are red radishes (Giusti & Wrolstad, 1996), red potatoes (Rodriguez-Saona, Giusti, & Wrolstad, 1999), red cabbages (Dyrby, Westergaard, & Stapelfeldt, 2001) and black carrots (Stintzing et al., 2002).

Black carrots are a good source of anthocyanin pigments. The anthocyanin content of black carrots was reported to be 1750 mg kg^{-1} fresh weight (Mazza & Miniati, 1993). Black carrots also contain high amounts of acylated anthocyanins. Stintzing et al. (2002) identified four major anthocyanins in black carrot extract and found 41% of anthocyanins to be acylated, namely cyanidin 3-sinapoyl-xylosyl-glucosyl-galactoside (27.5%) and cyanidin 3-feruloyl-xylosyl-glucosyl-galactoside (13.5%). Contrary to grape skin, black carrots contain low amounts of non-anthocyanin phenolics which can cause hazing and precipitation in clear fruit juices (Downham & Collins, 2000). Moreover, black carrot anthocyanins provide an excellent bright strawberry red shade at acidic pH values; therefore, black carrot juice can be a good choice for colouring fruit juices and nectars, soft drinks, conserves, jellies and confectionery (Downham & Collins, 2000). Furthermore, black carrot juice, like all fruit and vegetable juices, is considered as an ingredient when added to foods as a colorant. Therefore, black carrot juice does not require declaration with an E-number on food labels. Finally, since black carrots contain a high amount of nutraceutical components (Alasalvar, Grigor, Zhang, Quantick, & Shahidi, 2001), colouring of foods with black carrot juice may also provide a health benefit.

In the literature, there are only a few studies on black carrot anthocyanins. These studies are mostly concentrated on the anthocyanin composition of black carrots (Glassgen, Wray, Strack, Metzger, & Seitz, 1992; Kammerer, Carle, & Schieber, 2003; Narayan & Venkataraman, 2000). Our objective was to colour various non-anthocyanin-containing fruit juices and nectars with black carrot juice concentrate and to determine the stability of black carrot anthocyanins in these matrices during heating and storage.

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. Oranges, grapefruits, tangerines and lemons were purchased from a local market in Ankara. White grapes (cv. Narince) and apples (cv. Golden Delicious) were obtained from the Horticulture Department' s experimental orchards at Ankara University. Pineapple nectar was purchased from a local market while apricot and peach nectars were obtained directly from the fruit juice pilot plant in the Department of Food Engineering.

2.2. Sample preparation

Black carrots were washed in cold tap water, ground and pressed on a rack and cloth press (Bucher-Guyer, Niederweningen, Switzerland). After adjusting pH from 6.0 to 4.3 with 20% citric acid, the juice was depectinized with the enzyme Panzym P5 (Begerow, Langenlonsheim, Germany) at 50 °C for 2 h and filtered. The resulting clear juice was then concentrated to 64° Brix by a rotary low pressure evaporator. The rest of the juices and nectars were depectinized with Pectinex 3XL (Novo Nordisk, Dittingen, Switzerland).

Citrus juice was extracted using a household extractor (Moulinex T574, France) and filtered through cheesecloth to remove coarse particles. The juice was depectinized at 50 °C for 1.5 h. The depectinized juice was then clarified with 6 ml of 5% bentonite and 5 ml of 15% kizelsol at 50 °C for 30 min. Finally, the clarified juice was centrifuged at 6010*g* for 9 min and then filtered. Apples and grapes were pressed on the same press. The apple and grape juices, and apricot, peach and pineapple nectars were depectinized at 50 °C for 1 h and filtered. The filtered apple and grape juices were clarified with 3 and 12 ml of 5% gelatin, 7 and 10 ml of 5% bentonite and 5 and 0.5 ml of 15% kizelsol at 50 °C for 1 h, respectively. The clarified juices and the depectinized nectars were bottled and stored at −30 °C. Before the analyses, the juice/nectar samples were thawed at 4 °C overnight and then coloured with black carrot juice concentrate (1.5 g concentrate/100 ml juice). The coloured juice/nectar samples were used without further treatment for heating studies and pasteurized for storage studies. Before heating and storage studies, grape juice was filtered to remove the tartrate precipitates formed during storage.

2.3. Methods

2.3.1. Degradation studies

The thermal stability of anthocyanins from black carrots in various juices and nectars was studied at 70, 80

and 90 °C. The coloured juice (25 ml portions) was transferred into Pyrex tubes. The tubes were well capped to avoid evaporation and placed in a thermostatic water bath (Memmert, 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 anthocyanin content.

The storage stability of anthocyanins from black carrots in various juices and nectars was also studied at 4, 20 and 37 °C. The coloured juices and nectars (50 ml) were filled into glass bottles and the headspace was flushed with nitrogen. The bottles were then hermetically capped and pasteurized in a water bath at 85 °C for 15 min. The pasteurized juices/nectars were then transferred into a Sanyo MIR 153 model refrigerated incubator (Sanyo, Gunma, Japan) at 4 °C and a Memmert BE 400 model incubator (Memmert, Schwabach, Germany) at 20 and 37 °C.

2.3.2. Anthocyanin analysis

Total anthocyanin contents of samples were determined using the pH-differential method described by Giusti and Wrolstad (2001). For this purpose, aliquots of fruit juice matrices containing black carrot concentrate were brought to pH 1.0 and 4.5 and allowed to equilibriate for 1 h. The absorbance of each equilibrated solution was then measured at the wavelength of maximum absorption (λ_{max}) and 700 nm for haze correction, using a ThermoSpectronic Helios-α model spectrophotometer (ThermoSpectronic, Cambridge, England). The λ_{max} ranged from 527 to 530 nm for black carrot anthocyanins in coloured juices and nectars. The difference in absorbance values at pH 1.0 and 4.5 was directly proportional to total anthocyanin concentration which was calculated, based on cyanidin-3-glucoside (Alasalvar, Al-Farsi, Quantick, Shahidi, & Wiktorowicz, 2005) with a molecular weight of 445.2 and molar absorbance of 29,600 (Giusti & Wrolstad, 2001).

Visible spectra of samples were determined by scanning the absorption between 350 and 700 nm. Quartz cuvettes of 1 cm length were used and all measurements were carried out at 25 °C. Absorbance readings were made against distilled water as a blank.

2.3.3. Other analyses

Brix was measured at 20 °C using an automatic Atago Rx7000-α digital refractometer (Atago, Tokyo, Japan) and pH with a Inolab Level 1 pH meter (WTW, Weilheim, Germany). Titratable acidity was determined according to the method outlined by IFJU (1968) and expressed as "*g citric acid/100 ml juice*". Ascorbic acid content was determined using 2.6-dichlorophenolindophenol-xylene extraction method and expressed as "*mg/100 ml juice*" (Anonymous, 1951).

3. Results and discussion

3.1. General

Physical and chemical characteristics of the juices and nectars coloured with black carrot juice concentrate are presented in Table 1.

3.2. Anthocyanin degradation during heating

Thermal degradation of anthocyanins from black carrots in all juices/nectars followed first-order reaction kinetics at 70–90 °C (Fig. 1). Our results are in agreement with those from the previous studies reporting first-order reaction kinetics for the degradation of anthocyanins (Cemeroğlu, Velioğlu, & Işık, 1994; Culpepper & Caldwell, 1927; Garzon & Wrolstad, 2002). The first-order reaction rate constants (k) and half-lives ($t_{1/2}$), i.e., the

Table 1
Analytical data of juices and nectars coloured with black carrot juice concentrate

Sample	Brix	pH	Titration acidity[a] (g/100 ml)	Ascorbic acid (mg/100 ml)	Anthocyanin[b] (mg/l)
Juice					
Apple	18.7	3.88	0.46	2.23	35.5
Grape	26.2	3.58	0.53	1.85	41.1
Orange	12.1	3.38	1.27	55.4	40.1
Grapefruit	9.9	3.02	1.97	35.6	33.9
Tangerine	11.1	3.31	1.18	21.9	39.0
Lemon	9.4	2.52	6.60	48.5	29.9
Nectar					
Apricot	15.1	3.68	0.58	1.03	39.7
Peach	14.8	3.54	0.54	1.52	37.7
Pineapple	12.2	3.71	0.46	1.94	41.2

[a] As anhydrous citric acid.
[b] As cyanidin 3-glucoside.

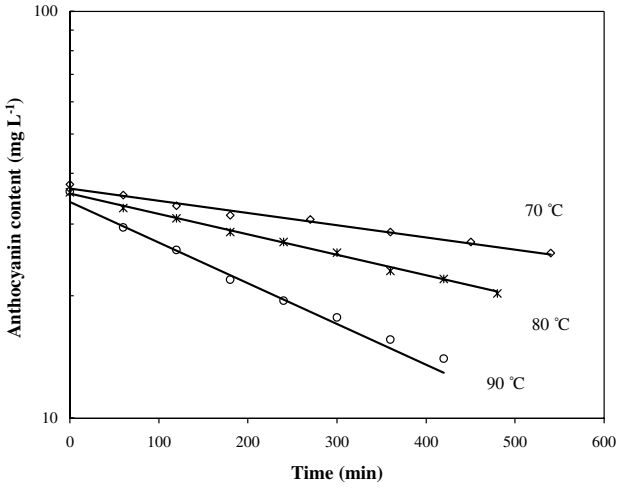

Fig. 1. Degradation of black carrot anthocyanins in apple juice during heating.

time needed for 50% degradation of anthocyanins, were calculated by the following equations:

$$\ln(C_t/C_0) = -k \times t, \quad (1)$$

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

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

Black carrot anthocyanins in apple and grape juices at 70 and 80 °C had a higher stability than those in citrus juices (Table 2). The higher stability of anthocyanins may be attributed to the much lower ascorbic acid content of apple and grape juices than the others (Table 1). As reported by several workers, ascorbic acid and its degradation products accelerate the degradation of anthocyanins (Poei-Langston & Wrolstad, 1981; Shrikhande & Francis, 1974). In fact, thermal stability of black carrot anthocyanins was the lowest in orange juice, which also contained the highest ascorbic acid among the coloured juices and nectars studied (Table 1). However, addition of ascorbic acid (30 mg ascorbic acid/100 ml juice) to the coloured apple and grape juices, and peach, apricot and pineapple nectars, did not affect the degradation rate of anthocyanins (data not shown). Kırca (2001) also observed a similar degradation pattern for the added ascorbic acid (50 and 100 mg/100 ml juice) in blood orange juice. Moreover, Choi, Kim, and Lee (2003) found that additional ascorbic acid did not significantly affect anthocyanin degradation in blood orange juice during storage at 4.5 °C.

The $t_{1/2}$ values of black carrot anthocyanins in orange juice were 12.5, 7.2 and 3.9 h at 70, 80 and 90 °C, respectively. Kırca and Cemeroğlu (2003) found that the $t_{1/2}$ values for the native anthocyanins of blood orange juice were 6.3, 3.6 and 1.5 h at the same temperatures. These results clearly showed that black carrot anthocyanins in orange juice had much higher stability than the native anthocyanins of blood oranges. Since blood oranges cannot be commercially processed into juices by conventional thermal processes due to the low stability of their anthocyanins (Maccarone, Maccarone, & Rapisarda, 1985), colouring yellow-pigmented orange juice with black carrot juice anthocyanins would produce stable red-coloured orange juice.

At 90 °C, the highest stability for black carrot anthocyanins was obtained in lemon juice, followed by apple juice, and grapefruit and tangerine juices. This result was surprising because lemon juice contained the highest ascorbic acid amount after orange juice. Contrary to lemon juice, anthocyanins in orange and grape juices exhibited the lowest stability at 90 °C. Compared to the juices, black carrot anthocyanins in nectars generally had slightly higher stabilities. Peach and apricot nectars showed exactly the same stabilities at all three temperatures.

There is no study found in the literature on the thermal stability of black carrot anthocyanins. However,

Table 2
Kinetic parameters for the thermal degradation of black carrot anthocyanin in various juices and nectars

Sample	$-k \times 10^3$ (min)$^{-1}$			$t_{1/2}$ (h)		
	70 °C	80 °C	90 °C	70 °C	80 °C	90 °C
Juice						
Apple	0.69 *(0.985)*[a]	1.15 *(0.997)*	2.30 *(0.989)*	16.7	10.1	5.0
Grape	0.69 *(0.987)*	1.38 *(0.996)*	2.99 *(0.997)*	16.7	8.4	3.9
Grapefruit	0.69 *(0.999)*	1.61 *(0.999)*	2.76 *(0.984)*	16.7	7.2	4.2
Lemon	0.92 *(0.988)*	1.61 *(0.984)*	2.07 *(0.935)*	12.6	7.2	5.6
Tangerine	0.92 *(0.997)*	1.61 *(0.997)*	2.76 *(0.983)*	12.6	7.2	4.2
Orange	0.92 *(0.996)*	1.61 *(0.997)*	2.99 *(0.983)*	12.6	7.2	3.9
Nectar						
Peach	0.69 *(0.988)*	1.15 *(0.988)*	2.53 *(0.991)*	16.7	10.1	4.6
Apricot	0.69 *(0.988)*	1.15 *(0.996)*	2.53 *(0.984)*	16.7	10.1	4.6
Pineapple	0.69 *(0.994)*	1.38 *(0.988)*	2.30 *(0.987)*	16.7	8.4	5.0

[a] Numbers in parentheses are the determination coefficients.

thermal stabilities of anthocyanins from different sources have been reported in both fruit juices and model systems. Calvi and Francis (1978) reported the $t_{1/2}$ values of 2.4 and 3.1 h for Concord grape anthocyanins in model systems containing sucrose and glucose at 90 °C, respectively. These values are lower than our $t_{1/2}$ values (3.95.6 h) for black carrot anthocyanins at the same temperature. Dyrby et al. (2001) found that $t_{1/2}$ values for anthocyanins in soft drink model systems (pH 3) at 80 °C were 193 h for red cabbage, 8 h for blackcurrant, 4 h for elderberry and 2 h for grape skin. Compared to these results, black carrot anthocyanins at 80 °C ($t_{1/2} = 7.2$–10.1 h) had a higher stability than elderberry and grape skin anthocyanins, similar stability to blackcurrant anthocyanins, but much lower stability than red cabbage anthocyanins. The authors attributed the excellent stability of red cabbage anthocyanins to the high degree of acylation (mostly diacylation) and glucosylation. Recently, Cevallos-Casals and Cisneros-Zevallos (2004) have compared the stabilities of anthocyanins from red sweet potato and purple corn with those from commercial black carrot and red grape colorants at 98 °C. They found higher stability of anthocyanins in black carrot and red sweet potato and attributed this to the higher acylation of these anthocyanins.

It should be pointed out that the stability of these anthocyanins from various sources was studied in model systems, while we determined the stability of black carrot anthocyanins in complex juice systems. Nevertheless, we also determined the stability of anthocyanins in citrate-phosphate buffer (pH 3) and found $t_{1/2}$ values of 25.1, 10.0 and 6.3 h at 70, 80 and 90 °C, respectively. These $t_{1/2}$ values are higher than those obtained for juices and nectars coloured with black carrot juice concentrate over the same temperature range studied (70–90 °C), reflecting the higher stability of black carrot anthocyanins in buffer solution. Compared to buffer solution, the lower stability of anthocyanins from black carrot juice may be attributed to the detrimental effect of sugar and ascorbic acid in juices/nectars (Dyrby et al., 2001). These findings clearly indicate that anthocyanin matrices have a notable effect on the stability. As indicated by Ribeiro, Ax, and Schubert (2003), food systems play a major role in pigment stability. In fact, Maccarone et al. (1985) found that only 25% of the anthocyanins from blood orange in a buffer solution (pH 3.2) was lost after three months of storage at 16–18 °C, while the anthocyanin loss reached up to 65% in blood orange juice under the same storage conditions. Moreover, in the above studies, pure anthocyanins chemically extracted from fruits and vegetables were used to colour model systems, while we coloured the juices and nectars directly with black carrot juice concentrate. Compared to the chemically extracted pigments, Rodriguez-Saona et al. (1999) found faster degradation of anthocyanins in juice model systems coloured with vegetable juice concentrates and attributed this to the complex composition of concentrates.

3.3. Anthocyanin degradation during storage

The degradation of black carrot anthocyanins in all the juices and nectars studied during storage at 4, 20 and 37 °C was also fitted to first-order reaction kinetics (Fig. 2). As expected, storage temperature had a clear effect on the degradation of anthocyanins. Storage at 37 °C resulted in a much faster degradation of anthocyanins than did refrigerated storage (4 °C). For example, $t_{1/2}$ values for black carrot anthocyanins in apple juice were 47.8 weeks at 4 °C and 1.7 weeks at 37 °C (Table 3). The effect of storage temperature on anthocyanin degradation was studied by Plocharski and Zbroszcyzk (1992), who showed that anthocyanin losses in black chokeberry juice were 47% at 4 °C and 81% at 20 °C after a year of storage. Similarly, Marti, Perez-Vicente, and Garcia-Viguera (2002) found that anthocyanin losses in pomegranate juice stored for two months were about 60% at 5 °C and 85% at 25 °C. The refrigerated temperature was found to increase the half-life of the red radish and red potato anthocyanins to more than one year (Rodriguez-Saona et al., 1999). Maccarone et al. (1985) showed that the degradation rate of anthocyanins in blood orange juice doubled for an increase of 10 degrees at 15–35 °C.

Black carrot anthocyanins generally showed the highest stability in tangerine and grape juices during storage at 4–37 °C (Table 3). At 20 °C, the $t_{1/2}$ values for black carrot anthocyanins in tangerine and grape juices were both 11.6 weeks. Rodriguez-Saona et al. (1999) reported a similar $t_{1/2}$ value for anthocyanins of red-fleshed potato juice concentrate (10 weeks) in a juice model system at 25 °C. However, they found a higher $t_{1/2}$ value for anthocyanins of red radish juice concentrate (16 weeks)

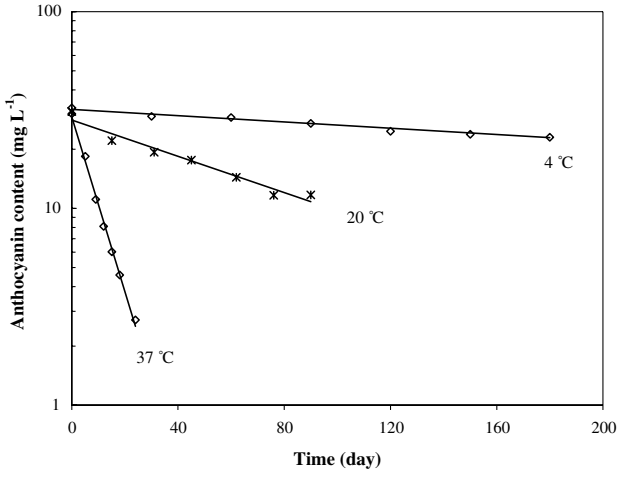

Fig. 2. Degradation of black carrot anthocyanins in pineapple nectar during storage.

Table 3
Kinetic parameters for the degradation of black carrot anthocyanin during storage in various juices and nectars

Sample	$-k \times 10^3$ (day)$^{-1}$			$t_{1/2}$ (week)		
	4 °C	20 °C	37 °C	4 °C	20 °C	37 °C
Juice						
Grape	0.69 *(0.971)*[a]	8.52 *(0.967)*	54.8 *(0.955)*	144	11.6	1.8
Tangerine	1.38 *(0.949)*	8.52 *(0.964)*	45.1 *(0.959)*	71.8	11.6	2.2
Grapefruit	1.84 *(0.998)*	10.59 *(0.921)*	44.2 *(0.875)*	53.8	9.4	2.2
Apple	2.07 *(0.953)*	8.75 *(0.979)*	59.9 *(0.989)*	47.8	11.3	1.7
Lemon	2.07 *(0.870)*	9.90 *(0.872)*	43.5 *(0.782)*	47.8	10.0	2.3
Orange	2.30 *(0.969)*	13.82 *(0.947)*	71.6 *(0.959)*	43.1	7.2	1.4
Nectar						
Peach	1.15 *(0.966)*	9.21 *(0.980)*	50.9 *(0.985)*	86.1	10.8	2.0
Apricot	1.38 *(0.985)*	9.90 *(0.961)*	61.3 *(0.994)*	71.8	10.0	1.6
Pineapple	1.84 *(0.975)*	10.59 *(0.958)*	102.0 *(0.996)*	53.8	9.4	1.0

[a] Numbers in parentheses are the determination coefficients.

in the same system. Similarly, the $t_{1/2}$ values of syrups coloured with red radish anthocyanin extract at a concentration of 60 and 120 mg anthocyanin per 100 ml syrup were found to be 29 and 33 weeks at room temperature (25 °C), respectively (Giusti & Wrolstad, 1996). The higher stability of red radish anthocyanins was attributed to the presence of diacylation as compared to the monoacylation of red-fleshed potato anthocyanins (Rodriguez-Saona et al., 1999). Much lower $t_{1/2}$ values at 25 °C were reported for pelargonidin-based anthocyanins in strawberry juice (12 days) and concentrate (5 days) by Garzon and Wrolstad (2002). The authors did not find any differences for $t_{1/2}$ values of acylated and non-acylated anthocyanins in either juices or concentrate. We found the lowest stability of black carrot anthocyanins in orange juice during storage at 4–37 °C (Table 3). At 20 °C, the $t_{1/2}$ value for coloured orange juice was 7.2 weeks as compared to 11.6 weeks for coloured tangerine and grape juices. Similar results were observed by Asafi (1995) who studied the stability of anthocyanins in various juices blended with sour cherry juice at 20 °C and reported that sour cherry anthocyanins were the least stable in orange juice. We also found that the stability of black carrot anthocyanins in nectars during storage at 4–37 °C was the highest in peach nectar and the lowest in pineapple nectar. As expected, storage at 37 °C resulted in a much higher anthocyanin degradation in all three nectars studied, especially in pineapple nectar. The calculated $t_{1/2}$ values at 37 °C were 2.0 weeks for peach nectar, 1.6 weeks for apricot nectar and only 1.0 week for pineapple nectar.

The losses of anthocyanins in coloured juices and nectars were found to be: 57% in apple, grape and tangerine juices, 59% in peach nectar, 63% in apricot nectar, 65% in pineapple nectar, 66% in grapefruit and lemon juices and 75% in orange juice after three months of storage at 20 °C. Spayd, Nagel, Hayrynen, and Drake (1984) determined the colour stability of apple and pear juices blended with anthocyanin containing juices and reported that the losses of anthocyanins in juices were 20% in black raspberry blend, 26% in Bing cherry blend, 31% in Concord grape blend and 42% in red raspberry blend after three months of storage at 25 °C. Similarly, the anthocyanin losses for the blends of apple juice were found to be: 24–34% with bilberry juice, 40–41% with raspberry juice, 42–59% with blackberry juice, 46–69% with blackcurrant juice and 50–56% with red currant juice after 4 months of storage at room temperature (Nani, Di Cesare, Rizzolo, & Picariello, 1993). The reported higher colour stabilities could have been due to the much higher pigment concentration. In fact, Spayd et al. (1984) blended apple and pear juices with 5%, 10% and 20% anthocyanin containing fruit juices, and Nani et al. (1993) blended apple juice with 10–27.5% berry juices. On the other hand, we added only a small amount of black carrot juice concentrate to the fruit juices/nectars (1.5 g concentrate/100 ml juice). Garzon and Wrolstad (2002) concluded that anthocyanin content should be specified when comparing colours or pigment stabilities of different anthocyanin systems.

3.4. Temperature dependence

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

$$k = k_0 e^{-E_a/RT}, \quad (3)$$

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

Compared to heating, higher E_a values during storage were obtained for the degradation of black carrot anthocyanins in coloured juices/nectars (Table 4). The E_a values ranged from 42.1 to 75.8 kJ mol^{-1} during heating at 70–90 °C and 65.9–94.7 kJ mol^{-1} during storage at 4–37 °C. At 70–90 °C, the calculated E_a values for col-

Table 4
Effect of temperature on the degradation of black carrot anthocyanin in various juices and nectars

Sample	E_a[a] (kJ mol^{-1})	Q_{10}		E_a[b] (kJ mol^{-1})	Q_{10}	
		70–80 °C	80–90 °C		4–20 °C	20–37 °C
Juice						
Grape	75.8[c]	2.0	2.2	94.7	4.8	3.0
Grapefruit	71.9	2.3	1.7	68.8	3.0	2.3
Apple	62.2	1.7	2.0	72.7	2.5	3.1
Orange	61.0	1.8	1.9	74.4	3.1	2.6
Tangerine	56.9	1.8	1.7	75.4	3.1	2.7
Lemon	42.1	1.8	1.3	65.9	2.7	2.4
Nectar						
Peach	67.1	1.7	2.2	82.0	3.7	2.7
Apricot	67.1	1.7	2.2	82.0	3.4	2.9
Pineapple	62.4	2.0	1.7	86.7	3.0	3.8

[a] At 70–90 °C.
[b] At 4–37 °C.
[c] Determination coefficients for E_a values were over 0.98.

oured juices/nectars (61.0–75.8 kJ mol^{-1}) are similar (except for lemon and tangerine juices) to that reported by Cemeroğlu et al. (1994) for anthocyanins in sour cherry juice (68.5 kJ mol^{-1}) and by Kırca and Cemeroğlu (2003) for anthocyanins in blood orange juice (73.6 kJ mol^{-1}) over the same temperature range. Similar to tangerine juice ($E_a = 56$ kJ mol^{-1}), Dyrby et al. (2001) reported E_a values of 50 and 56 kJ mol^{-1} for blackcurrant and elderberry anthocyanins in drink model systems at 25–80 °C, respectively.

Since high activation energy reactions are more sensitive to temperature changes, black carrot anthocyanins, during storage, were more susceptible to temperature elevation than during heating. Q_{10} values were also calculated to describe the sensitivity of heating and storage temperatures to temperature changes. Q_{10} values of 1.7–2.3 at 70–80 °C and 1.3–2.2 at 80–90 °C (heating), and 2.5–4.8 at 4–20 °C and 2.3–3.8 at 20–37 °C (storage) were obtained. Higher Q_{10} values for storage indicated that storage temperatures are much more sensitive to temperature elevations than are heating temperatures. Similar results were obtained for storage temperature of 4–20 °C, indicating that low storage temperatures (4–20 °C) are more sensitive to temperature elevations than are high storage temperatures (20–37 °C).

4. Conclusion

Results from this study clearly showed that anthocyanins from black carrot juice have good stability during both heating and storage in coloured fruit juices/nectars. The highest stability was obtained for apple and grape juices during heating at 70 and 80 °C. During storage at 4–37 °C, black carrot anthocyanins in tangerine and grape juices showed the highest stability. Storage temperature had a tremendous effect on the stability of black carrot anthocyanins in all coloured juices and nectars. As expected, a very fast degradation occurred in all coloured juices and nectars stored at 37 °C, whereas refrigerated storage resulted in much lower degradation of anthocyanins. The lowest stability was detected in orange juice during both heating and storage. Nevertheless, orange juice coloured with black carrot juice would be a good alternative for red-pigmented blood orange juice which cannot be easily transformed into commercial production due to its highly sensitive anthocyanins.

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