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Degradation kinetics of anthocyanins in blood orange juice and concentrate

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Abstract

Thermal and storage stabilities of anthocyanins in blood orange juice and concentrate were studied over the temperature range 70–90 °C and 5–37 °C. Analysis of kinetic data suggested a first-order reaction for the degradation of blood orange anthocyanins with the half-lives of 6.3 to 1.5, 3.4 to 0.7 and 2.0 to 0.4 h for 11.2, 45 and 69°Brix samples between 70 and 90 °C, respectively. At 5, 20 and 37 °C, the half-lives were between 55.7 and 2.1, and 115.7 and 3.1 days for 45 and 69 °Brix samples, respectively. Activation energies for solid content of 11.2–69 °Brix ranged from 73.2 to 89.5 kJ mol^{-1}.

Keywords: Anthocyanins; Degradation kinetics; Blood orange juice; Concentrate

1. Introduction

Anthocyanins are responsible for the attractive red colours of many fruits and fruit products. Much work has concentrated on the stability of anthocyanins due to the growing interest in widespread use of anthocyanins as natural food colorants (Mazza & Brouillard, 1987). However, due to their high reactivity, anthocyanins readily degrade and form colourless or undesirable brown-coloured compounds. Many factors affect the stability of anthocyanins, including temperature, pH, oxygen, enzymes, the presence of copigments and metallic ions, ascorbic acid, sulfur dioxide, sugars and their degradation products (Jackman, Yada, Tung, & Speers, 1987; Mazza & Miniati, 1993).

Some varieties of orange (*Citrus sinensis L., Rutaceae*), which are known as "blood oranges", contain anthocyanin pigments. Cyanidin 3-glucoside and an acylated anthocyanin, cyanidin 3-(4″-acetyl)-glucoside, constitute about 50 and 18% of the total anthocyanins of blood oranges, respectively (Mazza & Miniati, 1993). In a recent study, Krifi, Chouteau, Boudrant, and Metche (2000) isolated 10 anthocyanin pigments from blood orange juice and showed that cyanidin 3-glucoside was the major anthocyanin in blood oranges. However, blood orange anthocyanins are not very stable. During pasteurization and storage, the juice loses its bright red colour and gains a dirty-brown colour (Maccarone, Maccarone, & Rapisarda, 1985). Therefore, blood oranges cannot be processed into juice commercially by the fruit juice industry. Maccarone et al. (1985) studied the stabilization of anthocyanins in blood orange juice. They found that microwave pasteurization and addition of tartaric acid and glutathione improved the stability. They also found that complexation of anthocyanins with rutin and caffeic acid provided the highest stability.

Thermal degradation of anthocyanins has been studied for strawberry (Markakis, Livingstone, & Fellers, 1957), black raspberry (Daravingas & Cain, 1968), raspberry (Tanchev, 1972), Concord grape (Calvi & Francis, 1978), plum (Raynal & Moutounet, 1989) and sour cherry (Cemeroğlu, Velioglŭ, & Işık, 1994). In a recent study by Krifi et al. (2000), the degradation of anthocyanins in blood orange juice was investigated during storage at −18 °C for a few days and at 4 °C in nitrogen for 12 months. However, there are no kinetic data for the degradation of blood orange anthocyanins. Thus, the objective of this study was to determine the kinetic parameters for blood orange anthocyanins in both juice and concentrates during heating and storage at various temperatures.

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2. Materials and methods

2.1. Materials

Blood oranges (*Moro*) were obtained from Citrus Fruits and Greenhouse Research Center, Antalya. The juice was extracted using a household centrifugal extractor (Moulinex T574, France) and filtered through cheesecloth to remove pulp. The juice was depectinized with 2 ml l^{-1} Pectinex 3XL (Novo Nordisk, Dittingen, Switzerland) at 50 °C for 1.5 h. The depectinized juice was clarified with 0.3 g bentonite and 5 ml of 15% (v/v) kizelsol per litre of juice at 50 °C for 30 min. The resulting clarified juice was then filtered, bottled and stored at −30 °C until used for analysis. The clarified juice was also concentrated to 45 and 69 °Brix by rotary low pressure evaporator (BUCHI Rotavapor R-114 model, Fawil, Switzerland) at 80 °C.

2.2. Methods

2.2.1. Heat treatment

The thermal degradation of blood orange anthocyanins was studied at 70, 80 and 90 °C. The juice was heated in a three-necked round-bottom flask which was placed in a thermostatic water bath (Memmert, Schwabach, Germany) adjusted to the applied temperatures. At regular time intervals, samples of 25 ml juice were taken into pyrex tubes and rapidly cooled by plunging into an ice water bath. Concentrates of 45 and 69 °Brix were divided into 10-ml portions and placed in Pyrex tubes. The tubes were well capped to avoid evaporation and placed in a thermostatic water bath. Samples were removed from the water bath at regular time intervals and rapidly cooled by plunging into an ice water bath. The contents of heated and cooled tubes were analysed for anthocyanin content.

The storage stability of blood orange anthocyanins was studied in concentrates of 45 and 69 °Brix at 5, 20 and 37 °C. For 5 °C, a Sanyo MIR 153 model Refrigerated Incubator (Sanyo, Gunma, Japan) was used, while the storage at 20 and 37 °C was performed in a Memmert BE 400 model incubator (Memmert, Schwabach, Germany).

2.2.2. Determination of anthocyanins

Total anthocyanins were determined by a pH-differential method described by Fuleki and Francis (1968). The absorption spectrum was scanned from 350 to 600 nm. The wavelength of maximum absorption was 520 nm for blood orange juice anthocyanins. All absorbance readings were made against distilled water as a blank. Spectrophotometric measurements were carried out using a Unicam Helios α model spectrophotometer (Unicam, Cambridge, England). Calculations were based on cyanidin-3-glucoside with molecular weight of 445.2 and molar absorbance of 29600 (Wrolstad, 1976).

3. Results and discussion

3.1. Physical and chemical characteristics of juice

The results of some physical and chemical analyses of the clarified blood orange juice are shown in Table 1. The values agree well with the values reported in the literature for blood orange juice (Maccarone et al., 1985; Fallico, Lanza, Maccarone, Asmundo, & Rapisarda, 1996).

3.2. Kinetics of anthocyanin degradation during heating

The anthocyanin contents of blood orange juice and concentrates are plotted as a function of time on semi-log graphic paper (Figs. 1–3). The linear relationship indicates that thermal degradation of blood orange anthocyanins follows first order reaction kinetics. Previous studies have shown that thermal degradation of anthocyanins follows a first-order reaction (Calvi & Francis, 1978; Cemeroğlu et al., 1994; Daravingas & Cain, 1968; Markakis et al., 1957; Tanchev, 1972; Tanchev & Joncheva, 1973). The first-order reaction rate constants (k) and half-lives ($t_{1/2}$), i.e. the times needed

Table 1
Analytical data of blood orange juice

Brix	11.20
pH	3.44
Titration acidity[a] (g/100 ml)	1.08
Formol number (ml 0.1 N NaOH/100 ml)	19.5
Reducing sugars(g/l)	48.2
Total sugars (g/l)	88.6
Sucrose (g/l)	38.3
Ascorbic acid (mg/100 ml)	44.5
Anthocyanin[b] (mg/l)	87.4

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

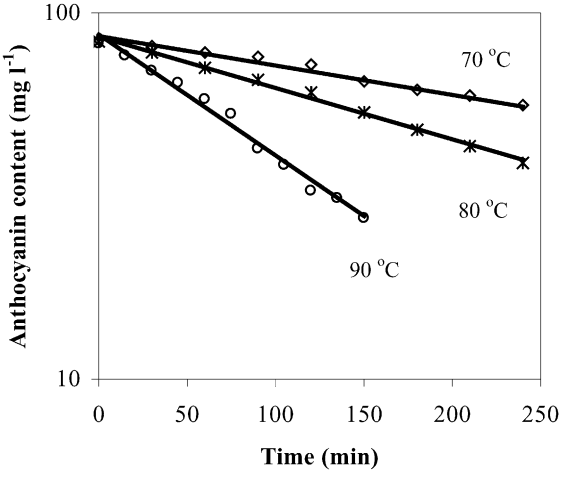

Fig. 1. Degradation of anthocyanins in blood orange juice (11.2 °Brix) during heating at 70, 80 and 90 °C.

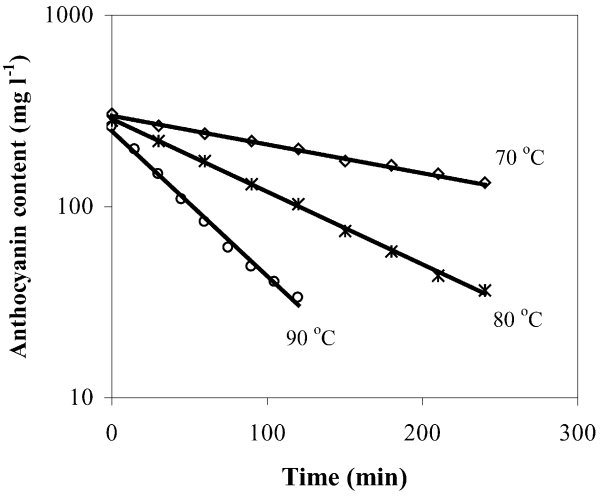

Fig. 2. Degradation of anthocyanins in blood orange juice concentrate (45 °Brix) during heating at 70, 80 and 90 °C.

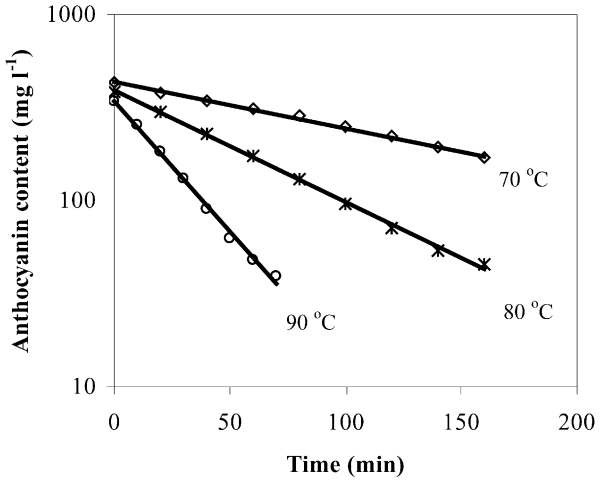

Fig. 3. Degradation of anthocyanins in blood orange juice concentrate (69 °Brix) during heating at 70, 80 and 90 °C.

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

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

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

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

The Arrhenius model was applied to describe the temperature dependence of anthocyanin degradation.

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

The kinetic parameters are shown in Table 2. As expected, anthocyanin degradation increased with increasing heating temperature and time. The k values (Table 2) varied from (1.84–7.60)$\times 10^{-3}$, (3.45–17.50)$\times 10^{-3}$ and (5.76–32.24)$\times 10^{-3}$ min^{-1} for 11.2, 45 and 69 °Brix

Table 2
Effect of temperature and solid content on the k, $t_{1/2}$ and E_a values of anthocyanin degradation in blood orange juice and concentrate

Solid concentrate (Brix)	Temperature (°C)	$k\ 10^3$ (min^{-1})	$t_{1/2}$ (h)	E_a (kJ mol^{-1})
11.2	70	1.84 (0.9803)[a]	6.3	
	80	3.22 (0,9945)	3.6	73.6 (0.9817)
	90	7.60 (0.9876)	1.5	
45	5	12.4[b] (0.9617)	55.7[c]	
	20	71.4[b] (0.9857)	9.7[c]	73.2 (0.9980)
	37	326[b] (0.9949)	2.1[c]	
	70	3.45 (0.9948)	3.4	
	80	8.75 (0.9984)	1.3	84.5 (0.9951)
	90	17.5 (0.9930)	0.7	
69	5	5.99[b] (0.9741)	116[c]	
	20	39.2[b] (0,9891)	17.7[c]	80.9 (0,9992)
	37	222[b] (0.9949)	3.1[c]	
	70	5.76 (0.9955)	2.0	
	80	13.8 (0,9978)	0.8	89.5 (1)
	90	32.2 (0.9959)	0.4	

[a] Numbers in parentheses are the determination coefficients.
[b] Day^{-1}.
[c] Days.

samples at 70, 80 and 90 °C, respectively. At the same temperatures, $t_{1/2}$ values (Table 2) ranged from 6.3 to1.5 h for juice, 3.4 to 0.7 h for concentrate of 45 °Brix and 2.0 to 0.4 h for concentrate of 69 °Brix, respectively. Cemeroğlu et al. (1994) showed that $t_{1/2}$ values for anthocyanin degradation were 54.3, 22.5 and 8.1 h in sour cherry juice, 24, 10.9 and 4.4 h in sour cherry concentrate of 45 °Brix and 13.1, 5.9 and 2.8 h in sour cherry concentrate of 71 °Brix at 60, 70 and 80 °C, respectively. Tanchev (1972) found that $t_{1/2}$ values for degradation of raspberry anthocyanins were 7.9, 3.4 and 1.5 h at 78, 88 and 98 °C, respectively. Compared to sour cherry and raspberry, blood orange anthocyanins are more susceptible to high temperatures.

At 70, 80 and 90 °C, the calculated activation energies (E_a) were 73.6, 84.5 and 89.5 kJ mol^{-1} for blood orange juice and concentrates of 45 and 69 °Brix, respectively (Table 2). Our E_a values are lower than those reported by Tanchev (1972) for raspberry anthocyanins (97.1 kJ mol^{-1}) and Tanchev and Joncheva (1973) for cyanidin 3-rutinoside and peonidin 3-rutinoside (99.2–118 kJ mol^{-1}) in a model system, but similar to those reported by Cemeroğlu et al. (1994) for sour cherry juice and concentrates of 45 and 71 °Brix (68.5, 75.9 and 80.1 kJ mol^{-1}, respectively).

As expected, the degradation of anthocyanins occurred at a faster rate in concentrates than in juices. This is because, when a product is concentrated, the reacting molecules become closer; thus the rate of chemical

reactions accelerates (Nielsen, Marcy, & Sadler, 1993). The losses of anthocyanins in juice were 14.4, 21.5 and 60.9% at the end of 120 min heating at 70, 80 and 90 °C, respectively. Compared to juice, a much higher loss of anthocyanins occurred in concentrates. After 60 min heating at 70, 80 and 90 °C, the losses were 21.7, 38.3 and 68.3% in concentrates of 45 °Brix and 27.6, 54.8 and 85.9% in concentrates of 69 °Brix, respectively. Similar trends were observed in sour cherry anthocyanins (Cemeroğlu et al., 1994).

3.3. Kinetics of anthocyanin degradation during storage

The degradation of anthocyanins in concentrates of 45 and 69 °Brix during storage was also fitted to first-order reaction kinetics (Figs. 4 and 5). The same reaction order was found for the anthocyanins in blackcurrant syrups (Skrede, 1985), in sour cherry concentrates of 45 and 71 °Brix (Cemeroğlu et al., 1994) and in blackcurrant nectar (Iversen, 1999). Similarly to juice, the degradation of blood orange anthocyanins in concentrates occurred faster with increasing storage temperature. The k values varied between $(12.4–326)\times10^{-3}$ and $(5.99–222)\times10^{-3}$ day^{-1} for 45 and 69 °Brix samples at 5, 20 and 37 °C, respectively (Table 2). The $t_{1/2}$ values at the same temperatures were calculated as 55.7, 9.7 and 2.1 days for 45 °Brix samples and 116, 17.7 and 3.1 days for 69 °Brix samples, respectively. Cemeroğlu et al. (1994) showed that the $t_{1/2}$ values for anthocyanin degradation in sour cherry concentrates of 45 and 71 °Brix varied from 14 to 310 and 11 to 356 days at the same temperatures, respectively. Moreover, the $t_{1/2}$ values for anthocyanin degradation during storage at 20 °C were 15–24 months in blackcurrant syrups (Skrede, 1985) and 165 days in blackcurrant nectar (Iversen, 1999). These observations clearly indicate that blood orange anthocyanins are less stable during storage.

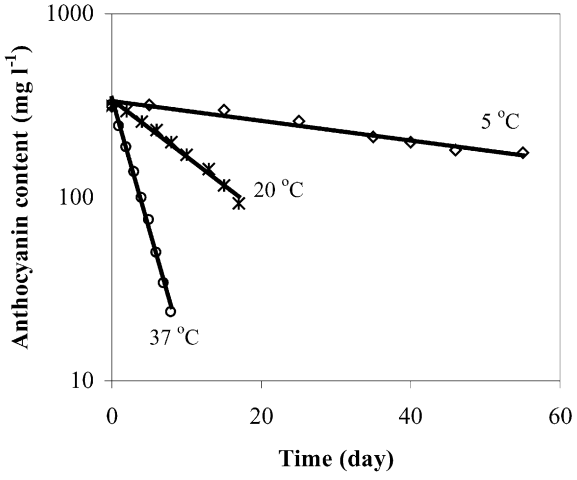

Fig. 4. Degradation of anthocyanins in blood orange juice concentrate (45 °Brix) during storage at 5, 20 and 37 °C.

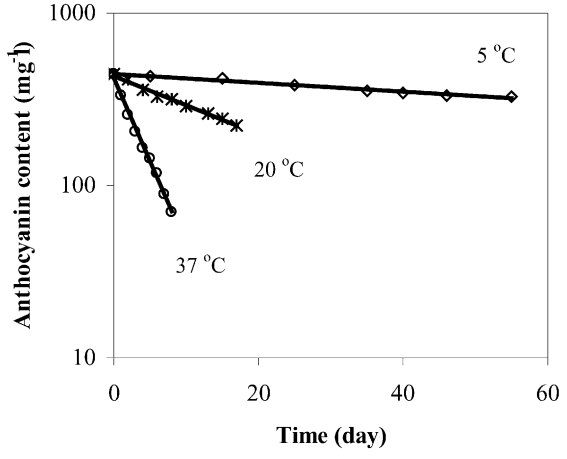

Fig. 5. Degradation of anthocyanins in blood orange juice concentrate (69 °Brix) during storage at 5, 20 and 37 °C.

Although the degradation of anthocyanins progressed at a faster rate with increasing solid content during heating, the anthocyanins, during storage, were degraded much more rapidly in 45 °Brix concentrates than in 69 °Brix concentrates. For example, the $t_{1/2}$ value at 20 °C was 9.7 days in 45 °Brix concentrate but 17.7 days in 69 °Brix concentrate. However, the calculated E_a value of 69 °Brix concentrate was higher (80.9 kJ mol^{-1}) than that of 45 °Brix concentrate (73.2 kJ mol^{-1}).

In conclusion, blood orange anthocyanins were found to be very susceptible to high temperatures. Also, commercial processing of blood orange into juice is not recommended unless stabilization of anthocyanins is provided by copigmentation or another way. Therefore, further studies are needed on the stabilization of anthocyanins in blood orange juice.

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