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ORIGINAL ARTICLE

Color stability and change in bioactive compounds of red beet juice concentrate stored at different temperatures

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Abstract In the present study, effect of storage temperature (25°, 35° and 45 °C) and light (with/without aluminum foil) on betalain content, color stability and bioactive compounds of concentrated red beet juice were investigated. Degradation of betalains and change in total phenolics content followed first-order kinetics while changes of L*, a*, b* and C* values and antioxidant capacity fitted zero-order kinetic. It was determined that the reaction rate constants of betacyanin and betaxanthin degradations in red beet juice concentrates increased with increasing storage temperature and time. The activation energies for betaxanthin degradation (E_a: 92.04–93.27 kJ mol^{-1}) in comparison with the activation energies for betacyanin degradation (E_a: 66.07–66.13 kJ mol^{-1}) in all samples demonstrate that susceptibility to temperature of betaxanthin is higher than that of betacyanin. According to L* and a* parameters it can be suggested that color stability in red beet juice concentrate stored with aluminum foil found better than that in the sample without aluminum foil. However, there was no significant difference ($p > 0.05$) between samples with and without foil as regards changes in total phenolics, betalains and antioxidant capacity. In addition, 25 °C can be proposed for providing betalain stability of red beet juice concentrates during storage.

Keywords Red beet juice concentrate · Storage · Betalain · Reaction kinetics · Antioxidant capacity · Visual color

Introduction

Red beets (*Beta vulgaris* L.) are nutritional vegetables that are primarily cultivated for its roots, which contain bioactive compounds, such as phenolics, cyclic amines, and various minerals (Amirasgari and Mirsaeedghazi 2015; Hwang et al. 2017). It is known that bioactive compounds that exhibit several beneficial properties, such as anti-inflammatory, antimicrobial, and antiviral effects, can also affect the proliferation of human tumor cells (Ravichandran et al. 2013; Mikołajczyk-Bator and Pawlak 2016). Moreover, the roots of red beets (beetroot) are a rich source of betalains, which have strong antioxidant properties (Azeredo 2009; Mikołajczyk-Bator and Czapski 2017). Betalains are reported to inhibit lipid peroxidation in very low amounts, might bind to human low-density lipoproteins, and could protect red blood cells against oxidative hemolysis (Sawicki et al. 2016). In addition, the antioxidant capacity of beetroot has been found to be related to phenolic compounds, which are responsible for preventing degenerative diseases and cancer (Raupp et al. 2011). Betalains, water-soluble nitrogen-containing pigments, display deep purplish-red in the beetroot (Azeredo 2009). These compounds are divided into two subclasses—purple betacyanins (Latin *Beta*, beet; Greek *kyanos*, blue), and yellow betaxanthins (Latin *Beta*; Greek *xanthos*, yellow) (Stintzing and Carle 2007; Azeredo 2009; Mikołajczyk-Bator and Pawlak 2016). A molecule of betacyanin consists

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of betalamic acid (BA) linked to a molecule of *cyclo*-3, 4-dihydroxyphenylalanine (*cyclo*-DOPA), and a molecule of betaxanthin consists of BA linked to an amino acid or amine (Herbach et al. 2006). Approximately 78 different structures of these compounds have been identified (Azeredo 2009; Ravichandran et al. 2013; Sawicki et al. 2016; Slimen et al. 2017).

Color is a significant characteristic of quality that can influence consumer preferences for foods; however, the use of synthetic colorants has decreased over the last decade because consumer preference for natural colorants has increased (Francis and Markakis 1989). Fruits and vegetables are good sources of natural colorants, such as betalains, anthocyanins, carotenoids, and chlorophylls. Betalains are also the major natural colorants that are applied to many food products, their use of which is approved by the European Union (labeled E-162). These compounds are particularly suited for coloring such foods as ice cream, wine, jams, marmalade, and yoghurt (Ravichandran et al. 2013; Solymosi et al. 2015; Sawicki et al. 2016); however, their stability is effected by temperature, pH, oxygen, light, water activity (a_w), enzymes, and some metal ions (Czapski 1990; Czapski et al. 1998). Betalains are less sensitive to pH and temperature than anthocyanins, which are appropriate only for acidic food products with a pH < 3.5. When pH is between 4.0 and 6.0, anthocyanin pigments lose their color (Mikołajczyk-Bator and Czapski 2017); however, betalains are stable within pH 3.0–7.0 (Herbach et al. 2006; Azeredo 2009; Klewicka 2012). Temperature is the most important factor that affects the stability of betalain during storage and processing, and it has been reported that betalain degradation increases with increasing temperature (Herbach et al. 2004a).

The objective of this study was to determine the effects of temperature and light on betalain content, total phenolics, antioxidant capacity, and color stability in red beet juice concentrate (RBJC) during storage. To avoid exposure to light, one-half of the samples stored in glass jars were covered with aluminum foil.

Materials and methods

Materials

RBJCs were obtained from a private company (Akdem Corporation) in Konya, Turkey and were stored in 105-mL glass jars at 25, 35, and 45 °C. One-half of the samples were wrapped with aluminum foil to prevent exposure to light. The other one-half of the samples were stored at the applied temperatures without foil. Because reactions occur slowly at low temperatures, the chosen storage periods were 105 d at 25 °C, 49 d at 35 °C, and 14 d at 45 °C. Samples from the jars heated to 25, 35, and 45 °C were taken at 15-, 7-, and 2-d intervals, respectively. Because betalain degrades faster at 45 °C, we chose 2 d as the sampling period. RBJCs were sampled equally at each applied temperature. All analyses were performed on two replicates.

Methods

Betalain (betacyanin and betaxanthin) pigment analyses

Betalain pigments were assayed using differential spectrophotometric methods according to Nilsson (1970). The principle for this method is based on color changes according to the pH of the red-pigmented betacyanin and the yellow-pigmented betaxanthin, which form the betalain structure. To measure the color changes, the samples were diluted with McIlvaine citrate–phosphate buffer solution at pH 4.5 (45.4 mL 0.2 M disodium phosphate [Na_2HPO_4] and 54.6 mL 0.1 M citric acid [$C_6H_8O_7$]), and the absorbance values were recorded at 488 nm for betacyanin and 532 nm for betaxanthin. The absorbances of the diluted samples were measured against air using the UV-1800 spectrophotometer (Shimadzu Corp., Kyoto, Japan). Betalain content was calculated according to Cai and Corke (1999). The contents of betacyanin or betaxanthin in the samples were determined according to the following formula:

$$\text{Betacyanin/Betaxanthin}\left(\text{mg L}^{-1}\right) = \frac{A \times DF \times MW \times 1000}{\varepsilon \times L},$$

where A is the absorption, DF the dilution factor, and l the path length (1 cm) of the cuvette. For quantification of betacyanins and betaxanthins, the molecular weights (MW) and molar extinction coefficients (ε) were applied (MW_{488} = 550 g mol^{-1}; ε_{488} = 60,000 L mol^{-1} cm^{-1}, respectively, for betacyanin and MW_{532} = 308 g mol^{-1}; ε_{532} = 48,000 L mol^{-1} cm^{-1}, respectively, for betaxanthin).

Analysis of total phenolic content

The total phenolic content was detected using a modified Folin–Ciocalteu colorimetric method (Shahidi et al. 2001). First, the concentrate was diluted with 0.5 mL distilled water mixed with 7 mL purified water and 0.5 mL Folin-Ciocalteu solution. After 3 min, 2 mL 20% Na_2CO_3 was added and the mixture was stirred and allowed to stand at 25 °C for 1 h in an incubator. By measuring the resulting blue intensity at 720 nm, the total amount of phenolic compounds contained in the sample could be calculated.

The results are expressed as the gallic acid equivalent per liter.

Trolox equivalent antioxidant capacity assay

Antioxidant capacity was estimated using the 6-hydroxy-2,5,7,8,-tetramethylchroman-2-carboxylic acid (Trolox) equivalent antioxidant capacity (TEAC) method and a UV/VIS spectrophotometer (Re et al. 1999). The 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) stock solution was prepared by reacting 7 mM ABTS stock solution with 140 mM potassium persulfate and incubating overnight in the dark for 12–16 h. A 30-µL sample and 10 µL Trolox were mixed with 1 mL ABTS·+ radical caption and the decrease in absorbance of the radical was measured at 734 nm for 6 min. Phosphate buffered saline (PBS) solution was used as a witness. The PBS solution was completed with distilled water to 1 L by adding 8.77 g NaCl into 0.1 M phosphate buffer to reach a pH of the antioxidant capacity calculated according to the ability of Trolox to scavenge ABTS·+ radical. Trolox was used as a standard compound, and the result was reported as mM TEAC/100 mL sample.

Color

The RBJC color characteristics were measured using the CIELAB color space (CIE L* a* b* color scale) with the Minolta CR-400 (Minolta Manufacturing Co., Osaka, Japan). The instrument was calibrated using a white tile and the samples were analyzed in triplicate.

Calculating kinetic parameters

The color losses and changes in antioxidant capacity were calculated using the following standard equation for a zero order-kinetics reaction:

$$C = C_0 - kt,$$

where C is the concentration at time t, C_0 is the concentration at time zero, k is the zero-order rate constant, and t is the storage time (d).

A first order-kinetics model to describe degradations of betacyanin, betaxanthin, and total phenolic contents in RBJCs is given in the following equation:

$$\ln C = \ln C_0 - kt,$$

where C is the concentration at time t, C_0 is the concentration at time zero, k is the first-order rate constant, and t is the storage time (d).

The temperature sensitivity of betacyanin and betaxanthin degradation, color loss, changes in total phenolic content, and changes in antioxidant capacity was determined using the Arrhenius equation (Labuza 1984) as follows:

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

where k is the rate constant, k_0 is the frequency factor, E_a is the activation energy (J mol^{-1}), R is the universal gas constant (8.314 J mol^{-1} K^{-1}), and T is the temperature in Kelvin (K).

Temperature quotients (Q_{10}) were calculated using the following equation:

$$Q_{10} = k_2/k_1^{10/(T_2-T_1)},$$

where k_1 is the rate constant at T_1, k_2 is the rate constant at T_2, T_1 and T_2 are the absolute temperatures in K.

Statistical analyses

All statistical analyses were performed using SPSS v. 20.0 (IBM Corp., Armonk, NY, USA). Significant differences among the samples were determined using the Duncan multiple range test with a 5% level of probability (Duncan 1955).

Results and discussion

The changes in betalain contents, visual color parameters, total phenolic content, and antioxidant capacity of RBJCs stored at different temperatures were investigated. RBJCs were stored in glass jars at different temperatures and with and without exposure to light. Some of the physical and chemical properties of RBJCs were examined before storage. The total soluble solids, titratable acidity, pH value, and water activity of RBJCs were determined to be 62.7°B×, 16.76 g 100 mL^{-1}, 4.12, and 0.824, respectively. In addition, antioxidant capacity and betacyanin, betaxanthin, and total phenolic content were detected as 151.4 mM Trolox 100 mL^{-1}, 1179 mg L^{-1}, 883 mg L^{-1}, and 4332 mg GAE L^{-1}, respectively.

Changes in color values during storage

The change in visual color was detected using the CIE L* a* b* color parameters. With an increase in temperature and time, L*, +b*, and C* values of RBJCs significantly increased ($p < 0.05$) with a corresponding significant decrease in +a* value ($p < 0.05$); however, hue values were observed to be variable. It was determined that L* of the samples increased by 2.1–7.4% based on the storage temperature and duration. The initial L* value of RBJC was 17.4, and the values were 18.70, 17.90, and 17.76, respectively, after storing RBJCs at 25, 35, and 45 °C in

glass jars without exposure to light. L* values for the samples stored in glass jars exposed to light at the various temperatures were 18.60, 17.79, and 17.71, respectively.

Zero-order reactions for the changes in L* in all samples are shown in Fig. 1. Kinetic parameters for the zero-order reaction of L* are presented in Table 1. E_a for L* was calculated using the Arrhenius equation. E_a values for the increase in L* in all samples with and without foil were calculated as 23 kJ mol^{-1} and 31 kJ mol^{-1}, respectively. Based on this result; we observed that L* in the samples without foil was more susceptible to temperature.

We also observed by the changes in +a* and +b* that the color of RBJCs during storage changed from deep purple to yellowish brown. The initial +a* value of RBJC was 0.11, but the values were 0.07, 0.06, and 0.04 and 0.06, 0.05, and 0.03, respectively, after storage at 25, 35, and 45 °C with foil and without foil. Percentage decreases in the +a* value in RBJCs in glass jars with foil at 25–45 °C were within the range of 36.4–63.6%. These percentages for +a* values of the samples in glass jars without foil stored at the same temperatures were calculated to be 45.5–72.7%. This result indicated that the samples stored in glass jars with foil retained their characteristic color more than those without foil. It is believed that the decrease in +a* value is from the degradation of betacyanin. Hence, this suggested strong and significant correlation coefficients (r = 0.901–0.973, $p < 0.01$) between +a* value and betacyanin content in RBJCs.

The zero order-kinetics reaction was the best fitting model for +a* value. The Q_{10} values for the decrease in +a* value in the samples with and without foil at temperature ranges 25–35 and 35–45 °C were determined as 2.0 and 5.4 and 2.6 and 3.9, respectively (Table 1). The E_a values for the changes in +a* value in the samples with foil (93.28 kJ mol^{-1}) and without foil (91.28 kJ mol^{-1}) were comparable. The difference in heat sensitivity between the samples with and without foil was found insignificant ($p > 0.05$). Slight and significant increases (48.7–75.7%) in +b* values in the samples with foil (1.10–1.25) and without foil (1.12–1.30) were observed compared to that in samples before storage (0.74). These increases also demonstrated that the changes in +b* value obeyed a zero order-kinetics reaction. The other kinetic parameters of +b* value are provided in Table 1.

The zero order-kinetics reaction was also determined for changes in C* value. The initial value of 0.74 increased to 1.07, 1.4, and 1.2 and to 1.06, 1.33, and 1.24 in the samples with and without foil at each applied temperature, respectively. E_a values were calculated as 95.61 kJ mol^{-1} and 100.95 kJ mol^{-1} for the changes in C* value in the samples with and without foil, respectively.

Özşen and Erge (2013) indicated that changes in the color of wild strawberries heated to 60, 70, 80, and 90 °C followed a first order-kinetics reaction. Tiwari et al. (2009) reported that the changes in a* and b* values in grape juice samples after ozone was applied reached a first order-kinetics reaction and that the decrease in L* value obeyed a zero order-kinetics reaction.

Total phenolic content

Initial total phenolic content in RBJC was 4332 mg GAE L^{-1} (Table 2). We observed that the total phenolic content significantly increased with an increase in storage time and temperature ($p < 0.05$). The increase in total phenolics in samples stored in glass jars with foil at 45 °C (k: 0.0093 d^{-1}) was 3.1–7.8 times higher than that in samples stored at 35 °C (k: 0.0030 d^{-1}) and 25 °C (k: 0.0012 d^{-1}), respectively. In addition, the enhancement in total phenolics in the samples without foil stored at 45 °C (k: 0.0085 d^{-1}) was 2.7–6.5 times higher than that in samples stored at 35 °C (k: 0.0031 d^{-1}) and 25 °C (k: 0.0013 d^{-1}), respectively (Table 1). The increase in total phenolics in RBJCs stored at the applied temperatures followed a first order-kinetics reaction. It has also been reported that total phenolic degradation followed the first

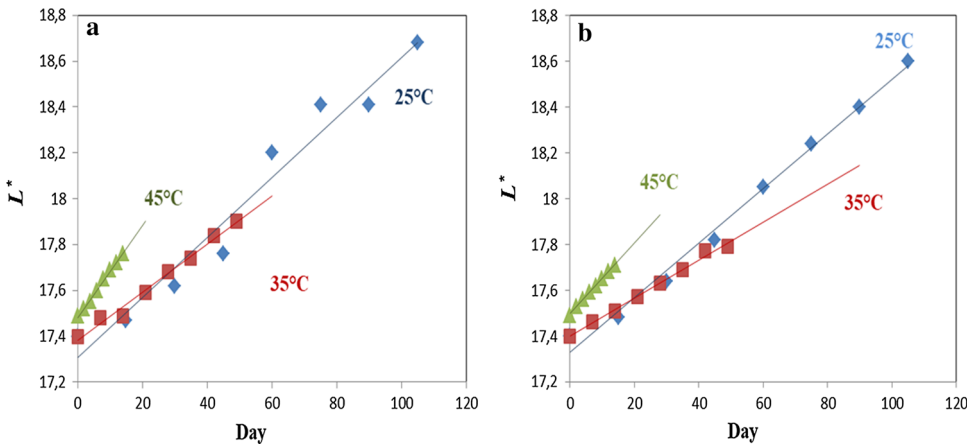

Fig. 1 Changes in the L* values in red beet juice concentrate (RBJC) stored in glass jars with (**a**) and without (**b**) foil at different temperatures

Table 1 Kinetic parameters for the changes in color values (L*, +a*, +b*, and C*), antioxidant capacity (AC), and total phenolic content (TPC) in red beet juice concentrates (RBJCs) stored in glass jars with/without aluminum foil at different temperatures

	Temperature T (°C)	Rate constant k *	Activation energy (kJ mol^{-1})	Q_{10}	
				25–35 °C	35–45 °C
With aluminum foil					
L*	25	0.0130 (0.9338)**	23	1.02	1.76
	35	0.0134 (0.9498)			
	45	0.0236 (0.9606)			
+a*	25	0.0005 (0.8726)	93.28	2	5.4
	35	0.0010 (0.9524)			
	45	0.0054 (0.9873)			
+b*	25	0.0031 (0.9594)	88.26	3.35	2.80
	35	0.0104 (0.9826)			
	45	0.0291 (0.9653)			
C*	25	0.0025 (0.8741)	95.61	4.96	2.27
	35	0.0124 (0.9719)			
	45	0.0281 (0.9525)			
AC	25	0.1984 (0.9707)	82.82	2.12	3.90
	35	0.4197 (0.9819)			
	45	1.6359 (0.9876)			
TPC	25	0.0012 (0.8951)	80.55	2.50	3.10
	35	0.0030 (0.7950)			
	45	0.0093 (0.9817)			
Without aluminum foil					
L*	25	0.0099 (0.9920)	31	1.18	1.86
	35	0.0117 (0.9950)			
	45	0.0218 (0.9165)			
+a*	25	0.0005 (0.9673)	91.28	2.60	3.9
	35	0.0013 (0.9584)			
	45	0.0051 (0.9810)			
+b*	25	0.0038 (0.9727)	82.03	2.87	2.80
	35	0.0109 (0.9918)			
	45	0.0305 (0.9673)			
C*	25	0.0025 (0.8792)	100.95	4.48	2.88
	35	0.0112 (0.9008)			
	45	0.0323 (0.9739)			
AC	25	0.2105 (0.9340)	81.96	2.22	3.62
	35	0.4681 (0.9682)			
	45	1.6960 (0.9805)			
TPC	25	0.0013 (0.9268)	73.88	2.39	2.74
	35	0.0031 (0.7834)			
	45	0.0085 (0.9784)			

Rate constant (k) units for L is L* d^{-1}; for +a* is +a* d^{-1}; for +b* is +b* d^{-1}; for C* is C* d^{-1}; for antioxidant capacity is mM Trolox 100 mL^{-1} d^{-1}, for total phenolic content is d^{-1}

**Determination coefficients (R^2) of degradation reactions

order-kinetics reaction model (Gonçalves et al. 2010; Özşen and Erge 2013).

Both the increasing and decreasing effects of storage conditions on total phenolics have been reported. Lewis et al. (1999) have reported an increase in total phenolic content of colored potato tubers during cold storage (4 °C); however, Duru et al. (2012) have reported that total phenolic content in rosehip nectars decreased significantly with increasing storage time and temperature.

Table 2 Contents of betacyanin, betaxanthin and total phenolic and antioxidant capacity in red beet juice concentrates (RBJCs) stored in glass jars with aluminum foil (W-AF) and without aluminum foil (WO-AF) at different temperatures

Temperature (°C)	Day	Betacyanin content (mg L^{-1})		Betaxanthin content (mg L^{-1})		Total phenolics content (mg GAE L^{-1})		Antioxidant capacity (mM Trolox 100 mL^{-1})	
		W-AF	WO-AF	W-AF	WO-AF	W-AF	WO-AF	W-AF	WO-AF
25	0	1179.4 ± 4.8^{a,*}	1179.4 ± 4.8^{a}	883.0 ± 1.4^{a}	883.0 ± 1.4^{a}	4332.1 ± 75.8^{c}	4332.1 ± 75.8^{c}	151.4 ± 2.5^{f}	151.4 ± 2.5^{c}
	15	1009.3 ± 28.8^{b}	1009.7 ± 20.8^{b}	720.0 ± 32.7^{b}	718.7 ± 26.0^{b}	4448.2 ± 50.5^{c}	4466.1 ± 101.0^{bc}	152.4 ± 1.1^{ef}	153.5 ± 3.2^{bc}
	30	930.4 ± 10.6^{c}	964.3 ± 36.4^{c}	659.3 ± 11.3^{c}	682.4 ± 30.9^{c}	4403.6 ± 12.6^{c}	4376.8 ± 113.6^{c}	154.3 ± 1.9^{e}	154.4 ± 9.1^{bc}
	45	807.2 ± 15.8^{d}	791.1 ± 52.1^{d}	580.4 ± 4.5^{d}	567.6 ± 47.2^{d}	4412.5 ± 50.5^{c}	4501.8 ± 126.3^{bc}	158.2 ± 0.1^{d}	158.8 ± 0.6^{b}
	60	780.6 ± 54.6^{d}	730.0 ± 21.0^{e}	553.8 ± 29.7^{e}	517.5 ± 20.6^{e}	4546.4 ± 202.0^{bc}	4680.4 ± 315.7^{abc}	159.4 ± 0.9^{d}	158.9 ± 0.1^{b}
	75	627.9 ± 10.4^{e}	641.7 ± 12.8^{f}	451.1 ± 14.4^{f}	458.5 ± 0.6^{f}	4742.9 ± 113.6^{ab}	4751.8 ± 25.3^{ab}	165.2 ± 2.0^{c}	168.6 ± 1.7^{a}
	90	279.6 ± 1.3^{f}	276.4 ± 2.8^{g}	396.9 ± 6.0^{g}	394.7 ± 4.0^{g}	4805.4 ± 88.4^{a}	4796.4 ± 37.9^{ab}	168.4 ± 0.7^{b}	170.1 ± 1.5^{a}
	105	246.1 ± 2.2^{f}	246.8 ± 3.2^{g}	348.5 ± 5.8^{h}	348.5 ± 9.0^{h}	4894.6 ± 12.6^{a}	4983.9 ± 113.6^{a}	170.9 ± 0.8^{a}	171.4 ± 0.6^{a}
35	0	1179.4 ± 4.8^{a}	1179.4 ± 4.8^{a}	883.0 ± 1.4^{a}	883.0 ± 1.4^{a}	4332.1 ± 75.8^{f}	4332.1 ± 75.8^{c}	151.4 ± 2.5^{f}	151.4 ± 2.5^{e}
	7	1034.5 ± 2.3^{b}	1042.3 ± 15.7^{b}	787.0 ± 2.2^{b}	791.2 ± 10.2^{b}	4582.1 ± 88.4^{de}	4671.4 ± 88.4^{bc}	152.3 ± 0.3^{f}	152.3 ± 1.2^{e}
	14	697.0 ± 21.8^{c}	694.2 ± 67.1^{c}	561.1 ± 16.3^{c}	545.4 ± 48.2^{c}	4492.9 ± 0.0^{ef}	4591.1 ± 101.0^{bc}	155.0 ± 0.1^{e}	157.1 ± 1.0^{d}
	21	651.8 ± 47.7^{d}	658.2 ± 7.8^{c}	500.2 ± 30.0^{d}	513.0 ± 8.6^{d}	4850.0 ± 25.3^{bc}	4983.9 ± 50.5^{ab}	160.3 ± 0.6^{d}	162.7 ± 0.6^{c}
	28	433.3 ± 21.8^{e}	491.8 ± 21.8^{d}	307.7 ± 9.4^{e}	360.0 ± 9.7^{e}	4733.9 ± 75.8 ^{cd}	4921.4 ± 505.1^{ab}	163.2 ± 0.8^{c}	165.7 ± 2.9^{bc}
	35	361.3 ± 6.7^{f}	371.5 ± 23.0^{e}	223.5 ± 19.2^{f}	226.5 ± 9.3^{f}	4983.9 ± 176.8^{ab}	5028.6 ± 63.1^{ab}	165.1 ± 0.8^{c}	166.5 ± 1.5^{b}
	42	330.7 ± 24.4^{fg}	333.7 ± 3.2^{ef}	185.5 ± 10.5^{g}	200.0 ± 5.5^{f}	4787.5 ± 12.6^{bcd}	4894.6 ± 12.6^{ab}	167.4 ± 1.9^{b}	168.6 ± 1.2^{b}
	49	317.3 ± 0.8^{g}	319.9 ± 5.2^{f}	174.1 ± 5.5^{g}	171.5 ± 7.4^{g}	5189.3 ± 138.9^{a}	5233.9 ± 101.0^{a}	171.1 ± 0.9^{a}	174.7 ± 2.7^{a}
45	0	1179.4 ± 4.8^{a}	1179.4 ± 4.8^{a}	883.0 ± 1.4^{a}	883.0 ± 1.4^{a}	4332.1 ± 75.8^{c}	4332.1 ± 75.8^{d}	151.4 ± 2.5^{g}	151.4 ± 2.5^{e}
	2	963.4 ± 32.8^{b}	1042.7 ± 12.5^{b}	748.9 ± 28.9^{b}	807.6 ± 11.5^{b}	4421.4 ± 101.0^{c}	4461.6 ± 56.8^{d}	156.0 ± 1.2^{f}	157.1 ± 1.8^{d}
	4	755.3 ± 18.1^{c}	776.0 ± 0.9^{c}	626.0 ± 10.9^{c}	638.1 ± 0.7^{c}	4466.1 ± 0.0^{bc}	4470.5 ± 18.9^{d}	159.0 ± 1.2^{e}	159.9 ± 1.7^{c}
	6	685.2 ± 18.2^{d}	722.3 ± 18.0^{d}	572.7 ± 14.9^{d}	596.4 ± 11.5^{d}	4617.9 ± 37.9^{b}	4608.9 ± 75.8^{c}	161.8 ± 1.4^{d}	165.3 ± 1.8^{b}
	8	573.8 ± 1.5^{e}	578.9 ± 6.0^{e}	462.3 ± 1.2^{e}	469.1 ± 7.6^{e}	4626.8 ± 75.8^{b}	4671.4 ± 12.6^{c}	165.7 ± 0.3^{c}	167.2 ± 1.0^{b}
	10	539.9 ± 13.8^{f}	548.2 ± 17.0^{f}	402.4 ± 25.2^{f}	424.2 ± 25.9^{f}	4796.4 ± 37.9^{a}	4698.2 ± 113.6^{bc}	169.8 ± 0.6^{b}	167.8 ± 0.3^{b}
	12	426.3 ± 19.1^{g}	435.7 ± 21.3^{g}	307.2 ± 23.9^{g}	316.3 ± 22.7^{g}	4832.1 ± 0.0^{a}	4832.1 ± 25.3^{ab}	172.6 ± 2.5^{a}	173.9 ± 1.2^{a}
	14	371.3 ± 19.99^{h}	384.6 ± 25.9^{h}	238.9 ± 37.0^{h}	254.0 ± 32.8^{h}	4930.4 ± 126.3^{a}	4903.6 ± 0.0^{a}	173.6 ± 1.8^{a}	176.4 ± 1.4^{a}

All values are the mean ± SD of three replicates

*Means within a column with different superscript letters are statistically different ($p < 0.05$) for each storage temperature

Antioxidant capacity

We observed that the initial antioxidant capacity of RBJCs (151.4 mM Trolox 100 mL^{-1}) significantly increased with storage time and temperature ($p < 0.05$) (Table 2). After storage at various temperatures, the percentage increases in the antioxidant capacities of RBJCs with and without foil were within the ranges of 12.9–14.6 and 13.2–16.5%, respectively. Vicente et al. (2006) have also reported that the antioxidant capacity of strawberry samples increased with increasing storage time and temperature. Özşen and Erge (2013) have also reported that the antioxidant capacity of wild strawberry pulp increased with storage temperature.

The increase in antioxidant capacity in this study followed a zero order-kinetics reaction. The reaction rate constants and other kinetic parameters for the increase in antioxidant capacity in RBJCs are provided in Table 1.

These results suggest that the increase in phenolic compounds might be responsible for the increased antioxidant capacity. In fact, high and important correlation coefficients ($r = 0.896$–0.996, $p < 0.01$) were found between total phenolic content and antioxidant capacity. It is also believed that the enhanced antioxidant capacity of RBJCs might be the result of the products of betalain degradation. Thus, Pedreño and Escribano (2001) have observed that the products of betalain degradation, such as BA and *cyclo*-DOPA (5-O-β-D-glucoside) contributed to antioxidant capacity.

Changes in betalain content (betacyanin and betaxanthin) during storage

Kinetic modeling of betalain degradation in RBJCs with and without aluminum foil and stored at given temperatures was investigated. It was determined that the changes in betacyanin and betaxanthin contents in RBJCs significantly decreased depending on storage time and temperature ($p < 0.05$). The initial betacyanin and betaxanthin values were 1179 mg L^{-1} and 883 mg L^{-1}, respectively (Table 2). Czapski et al. (2009) have detected the betacyanin content in RBJ as 620–1630 mg L^{-1} and in betaxanthin content as 310–950 mg L^{-1}. Georgiev et al. (2010) have found that the betalain content in the extracts obtained from beetroot were 47.11 mg g^{-1}. In that study, betacyanin and betaxanthin contents were 16.3 mg g^{-1} and 30.78 mg g^{-1}, respectively. It has been suggested that the varying values are related to the conditions under which the beets are cultivated and the methods by which the juice is processed.

Linear regression analysis confirmed that betacyanin and betaxanthin degradations in RBJCs kept in jars with foil (Fig. 2) and without foil (Fig. 3) followed first order-kinetics reactions. The following authors have stated that degradation of betacyanins in purple pitaya juice (Herbach et al. 2004b), of betalains at 90 °C in quinoa samples (Laqui-Vilca et al. 2018), and of beetroot betalains in cow's milk heated at 70–90 °C (Güneşer 2016) followed the first order-kinetics model.

Table 3 provides the rate constants, E_a, and Q_{10} values for betacyanin and betaxanthin degradations in RBJCs. We observed that the reaction rate constants for betacyanin and betaxanthin degradations in RBJCs increased with increasing storage time and temperature (Table 3). The results also show that betacyanin and betaxanthin degrade slower at 25 °C than at 35 °C and 45 °C over the same time period. For example, the betacyanin content in samples without foil was 1179.4 mg L^{-1}, which was reduced to 1009.7, 694.2, and 384.6 mg L^{-1} after $\sim$ 15 d at 25, 35, and 45 °C; therefore, the results suggest that RBJCs should be stored at low temperatures to protect the compounds from degrading.

The Q_{10} values indicate how many times a reaction rate increases for each 10 °C increase in temperature. These values for betacyanin and betaxanthin degradations in samples with foil were 1.99, 2.70, 4.36, and 2.43 within the ranges of 25–35 and 35–45 °C, respectively. Within the same temperature ranges for the losses of betacyanin and betaxanthin in samples without foil, the calculated Q_{10} values were 1.94, 2.78, 4.22, and 2.44, respectively. As seen in Table 3, betaxanthin degrades faster than betacyanin, especially at higher storage temperatures. The higher E_a for betaxanthin degradation in samples with or without foil also demonstrated that betaxanthin had a higher susceptibility to temperature than betacyanin.

E_a for the loss of betalain in Cactaceae fruit was 68.24 kJ mol^{-1} (Reynoso et al. 1997) and that for the degradation of beetroot betalains in cow's milk was 42.449 kJ mol^{-1} (Güneşer 2016).

Conclusion

This study indicates that storage time and temperature have an effect on betalains, visual color, antioxidant capacity, and total phenolic content in RBJCs. We observed that L*, +b*, and C* values significantly increased, while +a* value significantly decreased according to the zero order-kinetics reaction. Color stability (L* and +a*) in RBJCs stored with aluminum foil found better than that in the sample without aluminum foil. There was a significant decrease in betalain content in all RBJCs during storage, which followed the first order-kinetics model. The higher E_a for betaxanthin degradation in the samples also demonstrates that betaxanthin is more susceptible to higher temperatures than betacyanin. On the other hand, the

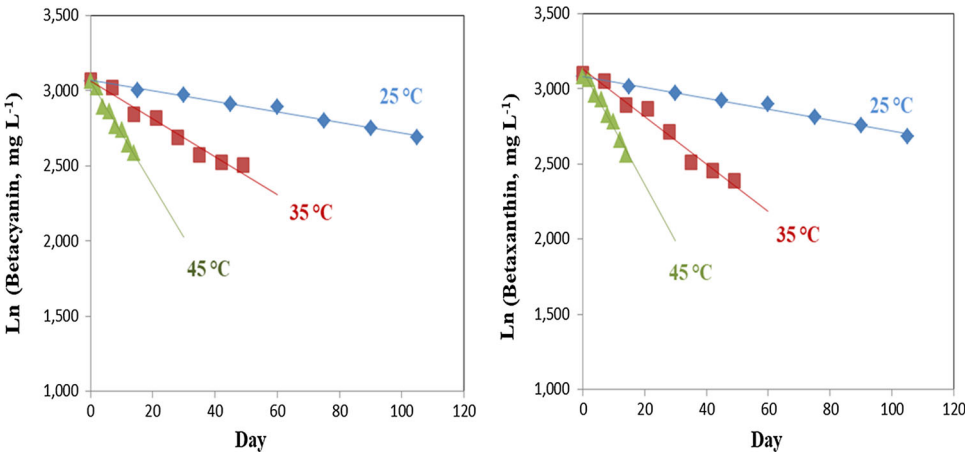

Fig. 2 Changes in the content of betacyanin and betaxanthin in red beet juice concentrate (RBJC) stored in glass jars with foil at different temperatures

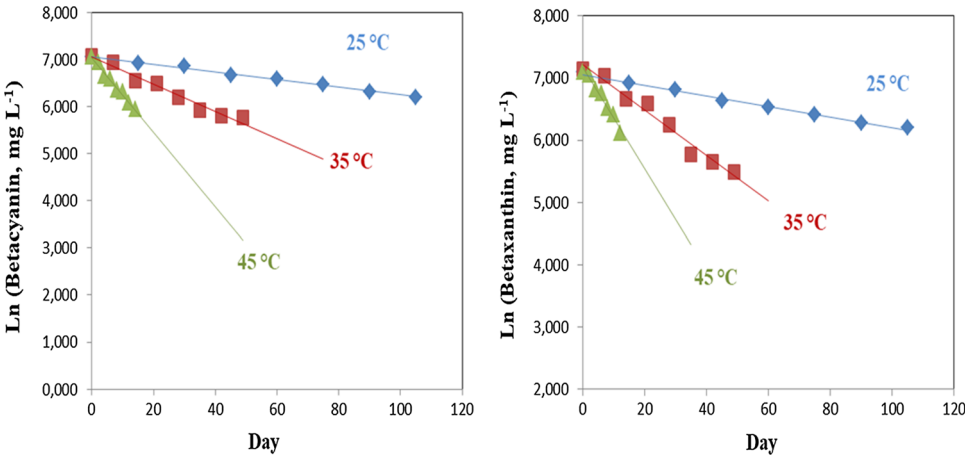

Fig. 3 Changes in the content of betacyanin and betaxanthin in red beet juice concentrate (RBJC) stored in glass jars without foil at different temperatures

Table 3 Kinetic parameters of betacyanin and betaxanthin degradations in red beet juice concentrates (RBJCs) stored in glass jars without/with aluminum foil at different temperatures

	Temperature T (°C)	Rate constant k (day^{-1})	Activation energy (kJ mol^{-1})	Q_{10}	
				25–35 °C	35–45 °C
With aluminum foil					
Betacyanin	25	0.0148 (0.8532)*	66.07	1.99	2.70
	35	0.0294 (0.9548)			
	45	0.0795 (0.9868)			
Betaxanthin	25	0.0085 (0.9875)	93.27	4.36	2.43
	35	0.0371 (0.9729)			
	45	0.0902 (0.9844)			
Without aluminum foil					
Betacyanin	25	0.0149 (0.8610)	66.13	1.94	2.78
	35	0.0289 (0.9670)			
	45	0.0802 (0.9853)			
Betaxanthin	25	0.0086 (0.9927)	92.04	4.22	2.44
	35	0.0363 (0.9754)			
	45	0.0885 (0.9813)			

*Determination coefficients (R^2) of degradation reactions

antioxidant capacity and total phenolic content of all samples significantly increased during storage ($p < 0.05$). Moreover, high and important correlation coefficients (r = 0.896–0.996, $p < 0.01$) were observed between total phenolic content and antioxidant capacity. Increases in antioxidant capacity can also be attributed to the products of thermal degradation of betalain pigment fractions. Because the betacyanin and betaxanthin in the samples at 25 °C degraded slower than those in samples at other storage temperatures during the same period, it is suggested that RBJCs be stored at low temperatures to prevent degradation. So, 25 °C can be proposed for providing betalain stability of RBJCs during storage.

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