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Kinetic Modelling of Betalain Stability and Color Changes in Yogurt During Storage

Onur Guneser

Department of Food Engineering, Engineering Faculty, Uşak University, 1 Eylül Campus, 64000, Uşak-Turkey

Key words: natural pigments, betalains, yogurt, reaction kinetics, color

Assessment of the storage stability of betalains added to food during processing is crucial to estimate the shelf-life of colored food products and the potency of natural food colorants. The stability of beetroot betalains in yogurt during storage was evaluated in this study. Kinetic experiments were conducted at storage temperatures of 4°C, 10°C, and 20°C. The relationships were also determined between the betalain degradation and lightness (L^*), redness (a^*), and yellowness (b^*). First-order kinetics was observed in the betalain degradation, and the changes in color parameters of the yogurt samples fitted zero-order kinetics. The activation energy required for the degradation of betalains and changes in L^*, a^*, and b^* was found as 104.9, 67.6, 76.5, and 86.1 kJ/mol, respectively. The half-life period of the degradation of red beet betalains was found as 51.43, 30.91, and 4.54 days at 4°C, 10°C, and 20°C, respectively. Multiple linear regression models were also established for betalain content and color parameters. There was a decrease in betalain content and a^* color value in the yogurt colored with a beetroot extract during storage. A significant positive correlation was found between pH, a^* value, and betalain content in yogurt, while a significant negative correlation was found between betalain content and L^* and b^* values. Further studies need to be carried out to reveal the relationship between color parameters and natural pigments in food systems.

INTRODUCTION

Since the last decade, natural pigments have attracted attention of the manufacturers in the food industry. The trend has shifted from artificial colorants to natural pigments owing to scientific studies regarding the potential risks of synthetic colorants to consumer health. At present, many scientific studies are being performed on natural pigments, and food manufacturers are trying to use them in food systems [Amchova *et al.,* 2015; Galaffu *et al.,* 2015]. Anthocyanins are the most studied natural pigments originating from plants, while betalains, carotenoids, chlorophylls, and curcumin are some other natural pigments that also offer beneficial health effects, such as preventing obesity [Martins *et al.*, 2016].

Betalains are heterocyclic derivatives of betalamic acids. They are divided into two categories, namely: betacyanins and betaxanthins. Betacyanins exhibit red to purple hues, while betaxanthins exhibit yellow to orange hues. Beetroot (*Beta vulgaris* L. ssp. *vulgaris*), colored Swiss chard (*B. vulgaris* L. ssp. *cicla*), amaranth (*Amarathus* sp.), cactus fruit (*Opuntia* sp.), pitayas (*Stenocereu* ssp.), and pitahayas (*Hylocereus undatus*) are the main plant sources of betalains. *Amanita muscaria* (fly agaric, a higher fungus) is also their natural source [Azeredo, 2009; Bárta *et al.,* 2020; Delgado-Vargas *et al.,* 2000; Gengatharan *et al.,* 2015]. Betalains have also gained interest owing to their health-promoting properties such as anti-atherogenic, anti-carcinogenic, anti-inflammatory, and hypolipidemic activities, along with colorant properties in food applications [Bárta *et al.,* 2020; Delgado-Vargas *et al.,* 2000; Moreno *et al.,* 2008]. Red beetroot is the commercial source of red colored betalains including betanin and isobetanin. Therefore, a beetroot extract or/and juice concentrate is used as a food colorant in many food products, such as dairy-based snacks, with E number 162 (E-162) [Azeredo, 2009; Herbach *et al.,* 2006].

Although the potential use of natural pigments is high, their application in foods is limited due to their low stability, weak tinctorial strength, strong interactions with food ingredients, and inability to match desired hues [Sigurdson *et al.,* 2017]. In this context, although betalains have certain pharmacological activities and color properties, it can be said that their main drawback is their strong earth-like aroma. Several types of studies are ongoing for their applicability in food systems using various techniques, such as co-pigmentation. Betalains as natural colorants in real food systems are less explored. A recent study [Gengatharan *et al.,* 2017] has shown the effects of pH and refrigerated storage on the stability of a colorant extract obtained from red pitahaya in yogurt. The degradation rate of betacyanin in yogurts containing the colorant extract at 14 days of refrigerated storage was 1.0%, while a loss of 1.6% betacyanin was observed in yogurt colored with a commercial

* Corresponding Author: Tel: +90 276 221 21 21 Ext: 2754;
E-mail: onur.guneser@usak.edu.tr (Dr. O. Guneser)

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colorant E-162. This study also showed that the extract from red pitahaya treated at pH 4 and 5 caused a lower reduction of betacyanins compared to E-162 in yogurt during ten weeks of refrigerated storage at 4°C.

Betalains are stable at pH ranging from 3 to 7 compared with anthocyanins, and are suitable coloring agents that can be stabilized by ascorbic acid [Sigurdson *et al.,* 2017]. Herbach *et al.* [2007] reported that the betacyanins present in purple pitaya (*Hylocereus polyrhizus*) could be easily stabilized by 1% ascorbic acid. In contrast, Karangutkar & Ananthanarayan [2021] found that 0.05% of ascorbic acid reduced betacyanin content in *Basella rubra* in a model beverage system during storage because of its pro-oxidant effect. Moreover, the addition of 5 mM (+)-catechin in a model beverage system was found to fulfill the maximum pigment retention at low temperature (4°C), and in the absence of light and oxygen.

Assessment of the thermal and storage stabilities of betalains added in real food systems during processing is crucial to estimate the shelf-life of colored food products and the potency of natural food colorants [Güneşer, 2016]. This study aimed to evaluate the stabilities of beetroot betalains in yogurt during storage at 4°C, 10°C, and 20°C using a chemical kinetics approach. The relationships between betalain content, color parameters, and pH during storage were determined using multiple linear regression and correlation analyses.

MATERIALS AND METHODS

Materials

Beetroot betalains used in the present study were acquired from a commercial natural liquid colorant from beetroot (*Wild Flavors,* ADM Wild GmbH, Eppelheim, Germany) that was obtained from NANTE Chemical Company (Istanbul, Turkey). Cow milk for yogurt production was obtained from a local producer (Usak, Turkey). The yogurt starter culture (*Büyüyo Yogurt Culture,* a mixed culture of *Lactobacillus delbrueckii* ssp. *bulgaricus* + *Streptococcus thermophiles* + *Lactobacillus acidophilus*) was obtained from Danem Dairy Company (Isparta, Turkey). All chemicals were of analytical/chromatographic grade and were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, MO, USA).

Production of yogurt with beetroot betalains

Yogurt was prepared according to the procedure of Yiğit *et al.* [2011]. Cow milk (6 L) was blended using a hand blender to get a homogeneous matrix. Then, the milk was distributed in three glass jars. The glass jars were placed in a water bath and heated to 85±2°C for 30 min. Then, they were placed in an ice-water bath for cooling. When the temperature of the glass jars had reached at 43±2°C, the beetroot colorant (3 g/L) and the yogurt starter culture (0.25 g/L) were added into each glass jar aseptically and then mixed by a hand blender at a medium speed (2 g force). The inoculated milk was poured into 100-g plastic cups (polypropylene) with lids (polyethylene terephthalate) and incubated at 43±2°C in an incubator (Nüve-ES 120, Ankara, Turkey) until pH= 4.7. After incubation, the yogurt samples were immediately shifted to refrigerated incubators for further experimentation. The procedures for the preparation of the yogurt samples were established based on the standard yogurt production steps [Tamime & Robinson, 2007]. Yogurt production was performed in duplicate. The amount of commercial beetroot colorant added into yogurt was determined by pre-coloring experiments and the recommendations of the producer. Moreover, in the Turkish Food Codex [TFC, 2013], the limit of beetroot colorant use was determined as a *quantum satis*. The inoculation rate of the yogurt culture was based on the recommendations of the Danem Dairy Company.

Storage experiment

The storage stability and changes in the color parameters of beetroot betalains in yogurt were examined at 4°C, 10°C, and 20°C for 60, 40, and 20 days, respectively, in refrigerated incubators (Nüve-ES 120, Ankara, Turkey and Memmert IPP500, Schwabach, Germany). The temperatures for yogurt storage were chosen by considering possible facilities such as transport, storage, and retail temperature conditions that consumers and manufacturers generally use for yogurt (4°C as storage temperature, 10°C as refrigerator temperature, and 20°C as cool ambient temperature). During storage, the yogurt samples were taken at regular time intervals (8-day for 4°C; 4-day for 10°C; and 2-day for 20°C) for chemical and color analyses, which were performed in duplicate for each storage temperature.

Titratable acidity, pH, and proximate analysis

Physicochemical properties of the yogurt samples, including pH, titratable acidity (g lactic acid/100g), total solids (g/100g), contents of fat (g/100g), protein (g/100g), and ash (g/100g) were determined by the methods described by Bradley *et al.* [1992].

Determination of betalain content in yogurts during storage

The betalain fraction was separated from yogurt samples using the method proposed by Gandía-Herrero *et al.* [2012]. In brief, 10 g of yogurt was centrifuged at 3075×*g* in a falcon tube at 10°C, and next the upper part was filtrated using a 0.45 μ PTFE syringe filter. Using this separation method, the recovery rate was >95%. Quantification of betalains (betanin+iso-betanin) in the yogurt fraction was performed by the HPLC with the external standard method [Naderi *et al*., 2012]. The Agilent 1260 HPLC system with Agilent multiple wavelength UV detector (Agilent Technologies Inc., Folsom, CA, USA) was used. Separation of betalains was carried out using a Zorbax SB-C18 column (Agilent, 4.6×250 mm, particle size of 5 μm). The mobile phase consisted of 0.5 mL/L trifluoroacetic acid solution and acetonitrile (90:10). The flow rate was kept constant at 1.0 mL/min, and the column temperature was maintained at 20°C for a total run. The detector was set at 540 nm for monitoring betalains. Betanin (product no: 901266, Sigma–Aldrich, St. Louis, MO, USA) was used as an external standard. The limit of detection, the limit of quantification, and repeatability were determined to be 7.12 mg/kg, 23.75 mg/kg, and 1.05 %, respectively. The content of betalains in yogurts was expressed as mg/kg yogurt.

Measurement of color parameters

The color parameters (L^* – lightness, a^* – redness and b^* – yellowness) of the yogurts were measured by using Minolta Cr-400 colorimeter (Minolta Co. Ltd., Osaka, Japan). A standard white plate was used for colorimeter calibration. Standard illuminant C and a standard observer angle (2°) were used for color measurements [ISO-CIE, 2008; Wrolstad & Smith, 2017]. The measurement was taken twice per sample.

Calculation of kinetic parameters

The storage stability of betalains and changes in color parameters of the yogurt samples during storage were evaluated using kinetic parameters (reaction order, reaction rate constants (k), and half-life period ($t_{1/2}$)). The effect of temperature on betalain degradation was studied and expressed by both activation energy (E_a) and temperature quotient (Q_{10}) [van Boekel, 2008].

Statistical analysis

Pearson's correlation and multiple regression analysis were performed to evaluate the relationship between the color parameters and betalain content of the yogurts [Sheskin, 2004]. SPSS software version 15.0 for Windows (IBM, Armonk, NY, USA) was used for the statistical analysis.

RESULTS AND DISCUSSIONS

Proximate composition, pH, and titratable acidity of the fresh yogurt

The stability of several natural pigments is affected by the physicochemical characteristics of food containing them. Therefore, the proximate composition of the fresh yogurt was determined, and the results are given in Table 1. The yogurt contained 13.13 g/100 g of dry matter and 2.50 g/100 g of fat, while about 3.01 g/100 g of protein and 0.85 g/100 g of ash on average. Its pH was about 4.53 and its titratable acidity was 0.81 g lactic acid/100 g. The chemical composition of yogurt may be affected by many factors, such as milk composition, presence of additives, process types, *etc*. The chemical composition of the fresh yogurt in the present study was typical of this kind of product, as supported by the results of previous studies [Özoğlu *et al.,* 2020; Yiğit *et al.,* 2011]. Özoğlu *et al*. [2020] investigated proximate compositions of homemade probiotic and commercial non-probiotic yogurts. They found that the pH values and dry matter contents of the yogurts were at 4.45–4.48 and 11.25–11.45 g/100 g, respectively. Protein and fat contents of the yogurts were found to range between 2.95 and 4.0, and between 3.01 and 3.70 g/100 g, respectively. Similarly, Farinde *et al.* [2009] reported 15.9 g/100 g of dry matter, 2.4 g/100 g of protein, and 0.4 g/100 g of ash contents for the commercial cow's milk yogurts sold in Nigeria. Hence, an average pH and acidity values of the yogurts were determined as 4.1 and 0.1 g lactic acid /100 g, respectively. The moisture content of the yogurt samples ranged from 83.3% in cow's milk

TABLE 1. Proximate composition, pH, and titratable acidity of the fresh yogurt.

Parameter	Value
pH	4.53±0.12
Titratable acidity (g lactic acid/100 g)	0.81±0.05
Dry matter content (g/100 g)	13.13±0.02
Fat content (g/100 g)	3.01±0.01
Protein content (g/100 g)	2.50±0.01
Ash content (g/100 g)	0.85±0.01

The values are expressed as means±standard deviation (SD).

Changes of yogurt pH and titratable acidity during storage

In the present study, the pH and titratable acidity of yogurts during storage were also monitored. The pH of yogurt decreased significantly during storage at all storage temperatures (Figure 1). The pH decrease was higher at 20°C than at other storage temperatures probably due to the higher metabolic activity of lactic acid bacteria at this temperature, as indicated by titratable acidity (Table 2). Increasing acidity in yogurt during storage is known as a post-acidification effect caused by the metabolic activities of lactic acid bacteria that produce lactic acid, and decreasing the shelf-life of yogurt [Shah, 2000].

Storage stability of betalains and changes in color parameters of yogurts

The chromatographic separations of betalains in the yogurts are shown in Figure 2. Two betalains were identified in the yogurts colored with beetroot colorants. The peak 1 with retention time of 10.53 min corresponds to betanin, while the second peak with retention time of 14.04 min to iso-betanin (Figure 2). Moreover, betanin and iso-betanin are predominant betalains in beet root, as mentioned before. The findings of the present study are in good agreement with the literature data [Azeredo, 2009; Herbach *et al.,* 2006]. The content of betalains in the yogurts stored at 4–20°C is provided in Figure 3. It was 305.18 mg/kg of the fresh yogurt and decreased during storage by 49.36%, 58.55%, and 95.65% at 4°C, 10°C, and 20°C, respectively.

The degradation of betalains followed the first-order reaction kinetics with high determination coefficients (R^2 = 0.983, 0.971 and 0.969 at 4°C, 10°C, and 20°C, respectively). Kinetic parameters for the degradation of betalains are given in Table 3. The k values were found in the range from -13.4×10^{-3}/day to -152.3×10^{-3}/day. The degradation rate of betalains in the yogurt samples increased with the elevation of storage temperature. This finding was confirmed by the half-life ($t_{1/2}$) values. Values $t_{1/2}$ for betalains in the yogurt samples were found to be 51.43, 30.91, and 4.54 days at 4°C, 10°C, and 20°C, respectively. Similarly to present findings, several studies have described the first-order kinetics of betalain degradation in different types of food under various storage and process conditions [Caldas-Cueva *et al.,* 2016; Kayın *et al.,* 2019; Tobolková *et al.,* 2020]. Kayın *et al.* [2019] reported the first-order kinetics for betacyanin and betaxanthin degradations in red beet juice concentrates stored in glass jars with and without aluminum foil at 25–45°C.

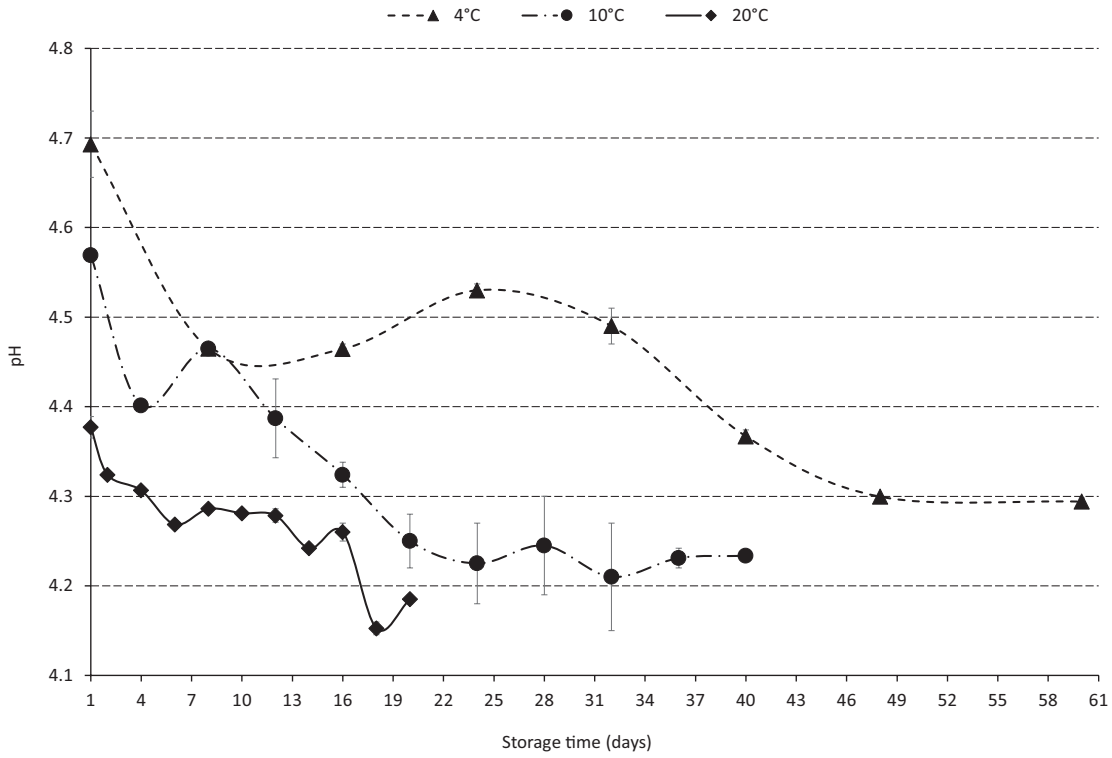

FIGURE 1. The pH value of the yogurts stored at 4–20°C.
The error bars represent the standard error.

TABLE 2. Titratable acidity (g lactic acid/100 g) of the stored yogurts.

Storage time (day)	Temperature (°C)		
	4	10	20
1	0.75±0.01	0.80±0.01	0.89±0.01
2	–*	–	0.98±0.05
4	–	0.84±0.03	0.97±0.01
6	–	–	0.96±0.01
8	0.82±0.02	0.92±0.01	1.01±0.01
10	–	–	1.03±0.01
12	–	0.99±0.01	0.99±0.01
14	–	–	1.02±0.01
16	0.92±0.02	0.91±0.02	0.96±0.04
18	–	–	1.03±0.02
20	–	0.92±0.01	1.01±0.01
24	0.91±0.13	0.92±0.01	–
28	–	0.99±0.01	–
32	0.90±0.01	0.95±0.01	–
36	–	1.01±0.01	–
40	0.86±0.01	0.90±0.04	–
48	0.90±0.01	–	–
60	0.91±0.01	–	–

*Analysis was not conducted on particular days. The values are expressed as means±standard error (SE).

They calculated k values reaching 14.8×10^{-3}/day, 29.4×10^{-3}/day, and 79.5×10^{-3}/day for betacyanin degradation at 25°C, 35°C, and 45°C, respectively, whereas low k values, such as 8.5×10^{-3}/day, 37.1×10^{-3}/day, and 90.2×10^{-3}/day, were calculated for betaxanthin degradation at the same storage temperatures.

In another study by Tobolková *et al.* [2020], the first-order reaction was observed for the degradation of both betacyanins and betaxanthins in apple-beetroot juice stored at 2°C, 7°C, and 20°C. The k values for betacyanin and betaxanthin degradations ranged from 7.3×10^{-3}/day to 47.1×10^{-3}/day and from 6.4×10^{-3}/day to 28.5×10^{-3}/day, respectively, which were lower than those observed in the present study. Similarly, Caldas-Cueva *et al.* [2016] reported that the degradation of betacyanins of an ayrampo (*Opuntia soehrensii*) seed extract and a red beet extract during the storage at 4°C and 25°C, at pH 4.5 followed the first-order reactions kinetics. The stability of betacyanins in the ayrampo seed extract was higher than that in the red beet extract at both temperatures tested. Color retention in yogurts containing the ayrampo seed extract and the red beet extract was not influenced by fat content, and the ayrampo seed extract showed better color retention over 4-week storage at 4°C compared with the red beet extract. Contrary to present findings, Moreno *et al.* [2007] reported the zero-order reaction for the degradation of betalains from tuna (*Opuntia elatior* Miller) and beetroot (*Beta vulgaris* L.) in four different citrus beverage formulations during storage at 7°C, with k values between 12.4 and 18.1 g/100 mL×day.

The differences in findings on betalain degradation could be attributed to the differences in the structure

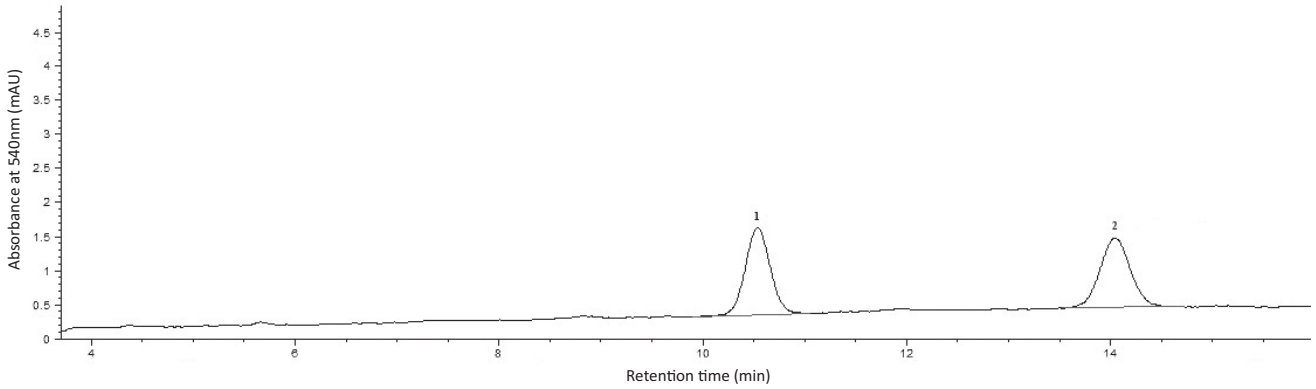

FIGURE 2. HPLC separation of the betalain fraction from yogurt with a red beet colorant. Peaks number 1 and 2 correspond to betanin and iso-betanin, respectively.

of individual betalains, food matrix and product formulation, process conditions, and storage temperatures [Azeredo *et al*., 2009; Khan, 2006]. Many types of reactions, such as hydrolysis, drive the degradation of betalains [Manchali *et al*., 2013]. Each reaction that has different responses regarding chemical kinetics occurs by various chemical mechanisms and depends on many factors. Betalains are considered as heat-labile pigments. Many researchers have reported [Herbach *et al*., 2004, 2007; Kayın *et al*., 2019] that their stability decreased by increasing temperature depending on pigment concentration, temperature level, exposure time of heating, or the presence of oxygen. It has been emphasized that betalains are considerably degraded between 50 and 75°C [Herbach *et al.*, 2006; Manchali *et al*., 2013]. They may be also degraded by isomerization at low temperatures, and their chemical structure and color change as a result of isomerization reactions [Herbach *et al.*, 2006]. Especially, betalains are transformed to the decarboxylated, dehydrogenated, or glycoside derivatives at high temperature. For instance, betanin (red) is conversed to neobetanin, which has yellow color, by dehydrogenation reaction, while 15-decarboxy-betanin (red), 17-decarboxy-betanin (orange-red), and cyclodopa-5-*O*-glycoside (colorless) are formed by the decarboxylation of betanin [Azeredo, 2009; Herbach *et al.*, 2006; Khan, 2016; Manchali *et al.*, 2013]. Fermentation was also determined to affect the stability of betalains. According to Czyżowska *et al.* [2006], betanidin and isobetanidin can be formed by izomerization in red beet juice during lactic acid fermentation. In addition, ratios of betanin+isobetanin/betanidin+isobetanidin, isobetanin/betanin, neobetanin/betanin, and vulgaxanthin I/betanin were found to change in the fermented red beet juice depending on the variety of beet processed (Czerwona Kula and Chrobry). The authors reported that these changes could be due to the activity of β-glucosidase sourced by lactic acid bacteria and the activities of certain

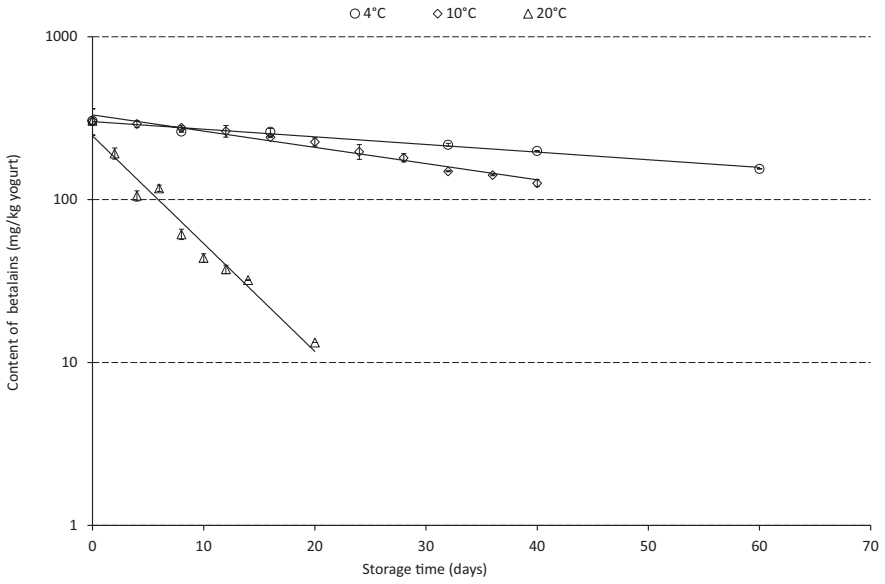

FIGURE 3. Betalain content in the yogurts stored at 4–20°C.
The error bars represent the standard error.

TABLE 3. The kinetic parameters determined for betalain degradation and for color parameter changes of the yogurts during storage.

Parameter	Temperature (°C)	Reaction order	Reaction rate constant (k)	$t_{1/2}$ (day)	Activation energy (E_a) (kJ/mol)	Q_{10}	
						4–10°C	10–20°C
			$\times 10^{-3}$ (1/day)				
Betalain content	4	First-order	-13.4±0.1 (0.983)[a]	51.43	104.9±0.1 (0.966)	2.43	6.61
	10		-23.0±0.9 (0.971)	30.91			
	20		-152.3±0.1 (0.969)	4.54			
			$\times 10^{-3}$ (L^*/day)				
L^*	4	Zero-order	28.8±1.6 (0.982)	-[b]	67.6±4.8 (0.988)	2.13	3.56
	10		45.5±1.7(0.952)	-			
	20		162.1±8.7 (0.852)	-			
			$\times 10^{-3}$ (a^*/day)				
a^*	4	Zero-order	-53.3±0.8 (0.958)	-	76.5±0.6 (0.994)	2.63	3.35
	10		-95.6±7.5 (0.982)	-			
	20		-321±1.4 (0.948)	-			
			$\times 10^{-3}$ (b^*/day)				
b^*	4	Zero-order	34.9±1.2 (0.906)	-	86.1±1 (0.990)	3.15	3.76
	10		69.7±6.5 (0.958)	-			
	20		262.6±1.9 (0.924)	-			

[a]Numbers in parentheses are determination coefficient (R^2). [b]The value was not calculated due to zero-order reaction. L^*: lightness, a^*: redness, b^*: yellowness, $t_{1/2}$: half-life, Q_{10}: temperature quotient. The values are expressed as means±standard deviation (SD).

enzymes including dehydrogenase, polyphenoloxidases, or peroxidase, which were found in the red beet juice medium [Czyżowska *et al*., 2006].

Unlike the changes in betalain content of the yogurt samples, changes in L^*, a^*, and b^* color parameters followed the zero-order kinetics during storage (Figure 4, Table 3). The L^* and b^* values increased, while the a^* values decreased during yogurt storage at all storage temperatures. Furthermore, k values determined for the changes in L^*, a^*, and b^* values were found in the range between 28.8×10^{-3} and 162.1×10^{-3} L^*/day; -53.3×10^{-3} and -321×10^{-3} a^*/day; as well as 34.9×10^{-3} and 262.6×10^{-3} b^*/day, respectively. According to this, the reaction rate determined for changes in the a^* values of the yogurts stored at all temperatures was higher than those determined for the L^* and b^* values (Table 3). This can be attributed to the changes in chemical structures and spectral properties of betalains as a result of several reactions. Indeed, degradation of betalains led to an increase in L^*, b^*, and hue angle values and to a decrease in a^* value, as commonly reported in previous research [Fernández-López *et al*., 2013; Gandia-Herrero *et al*., 2010; Herbach *et al*., 2004]. Different UV absorption and reflectance properties were reported for decarboxylated, dehydrogenated, or glycoside derivatives from betalains in different food matrices and solvents. According to this, betanin from red beet displays a maximum UV visible absorbance at 538 nm, while its dehydrogenated derivatives (neobetanins) have the maximum UV absorbance at 477 nm [Herbach *et al*., 2004].

Although several works are available on the kinetics of changes in the color parameters of anthocyanins in various foods [Reyes & Cisneros-Zevallos, 2007; Roidoung *et al.,* 2017], studies regarding the kinetics of color changes of betalains are sparse. Kayın *et al.* [2019] reported that L^*, a^*, and b^* value changes in a red beet juice concentrate stored at 25°C, 35°C, and 4°C followed the zero-order kinetics with k values lower than that found in the present study. Sonar *et al.* [2019] reported that L^*, a^*, and b^* values of beetroot puree packed in polymeric films with different oxygen transmission rates decreased during storage at 7°C, and the changes of overall color difference (ΔE) of beetroot puree were found to follow the zero-order reaction kinetics. In a study by Narkprasom *et al.* [2012], the first-order kinetics was reported for the degradation of Hunter a value of *Djulis* extract (*Chenopodium formosanum* Koidz.) in model systems containing various ethanol concentrations (0%–60%) during storage at 20–50°C. Similarly, Chandran *et al.* [2014] reported that the degradation of Hunter a/b values of beetroot at 50–120°C in an isothermal heating condition followed the first-order kinetics.

Activation energy (E_a) and temperature quotient (Q_{10}) values of betalain degradation and changes of the color parameters of the stored yogurts

The temperature dependency of the betalain degradation and color changes in the yogurt samples were expressed by E_a and Q_{10} values. The E_a value for the degradation of betalains in the yogurts during storage at 4–20°C was 104.9 kJ/mol,

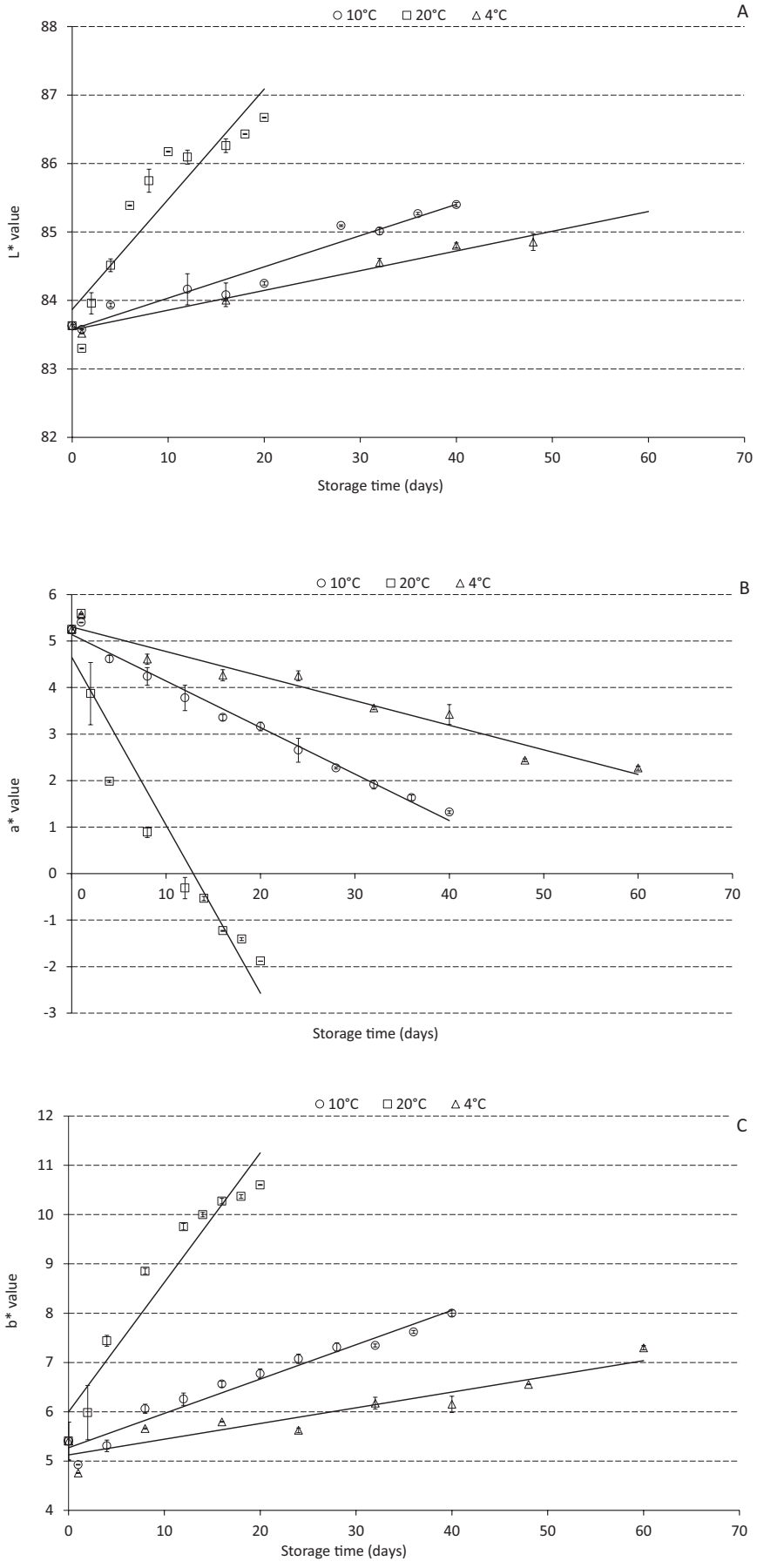

FIGURE 4. Color parameters – L^* – lightness (A), a^* – redness (B), and b^* – yellowness (C) – of the yogurts stored at 4–20°C. The error bars represent the standard error.

while calculated Q_{10} values were 2.43 and 6.61 at 4–10°C and 10–20°C, respectively (Table 3). Based on these results, it can be interpreted that the reaction rate for betalain degradation in the yogurts is more influenced by the temperature change from 10°C to 20°C compared with the temperature change from 4°C to 10°C. The E_a values denoting the color changes were 67.6, 76.5, and 86.1 kJ/mol for L^*, a^*, and b^* color parameters, respectively. Due to low E_a values, the changes in L^* and a^* values were more affected by the temperature elevation than b^* value. Indeed, Q_{10} values of the changes in b^* value were found to be higher than those of L^* and a^* values (Table 3).

Various E_a values for the betalain degradation and color changes in food systems have been reported [Das *et al.,* 2019; Kayın *et al.,* 2019; Siow & Wong, 2017]. In a study by Siow & Wong [2017], E_a values were calculated as 92.817 and 82.953 kJ/mol for the degradation of betacyanins in red-fleshed dragon fruit (*Hylocereus polyrhizus*) juice and its concentrate at 25°C, 37°C, and 45°C, respectively, and were lower than E_a value observed in the present study. Similarly, Kayın *et al.* [2019] reported lower E_a values, such as 66.07 and 93.27 kJ/mol, for betacyanin and betaxanthin degradations in a red beet juice concentrate stored in glass jars at 25–45°C. Those researchers have also reported E_a values at 23.00, 93.28, and 88.26 kJ/mol for L^*, a^*, and b^* color parameters, respectively [Kayın *et al.,* 2019]. Similarly to the present findings, Das *et al.* [2019] reported E_a values such as 119.75 and 125.34 kJ/mol for the degradation of betacyanins extracted from red amaranth using water and 50% ethanol at pH 1 and 3, respectively, at storage temperatures of 4°C and 30°C. In turn, the E_a value of 37.54 kJ/mol was reported by Chandran *et al.* [2014] for the changes of Hunter *a/b* value in beetroot heated at 50–120°C.

Relationships between the pH value, betalain content, and color parameter values in yogurts during storage

Unlike anthocyanins, betalains are color-stable in a food matrix in a wide range of pH values. The maximum UV absorption and color spectrum of betalains do not change significantly at pH from 3 to 7. According to Fu *et al*. [2020], the optimal stability of betalains was reached at pH 4–6. In the present study, the pHs of the yogurts ranged from 4.15 to 4.69 (Figure 1). Therefore, the color stability of betalains in the yogurts could be expected. However, changes in the color parameters were observed during storage (Figure 4). As explained earlier, these results could be related to the type of degradation, molecular interactions of betalains with the structural components of the yogurt matrix, and others factor, such as dissolved oxygen concentration, content of metal cations content, and enzymes, apart from the pH. Moreover, several types of acids and their amounts have different effects on the color of betalains [Khan, 2016]. In the present study, the titratable acidity (g lactic acid/100 g) of the yogurt samples increased gradually during the storage depending on the storage temperature (Table 2).

Synthetic or natural color pigments that are found in foods have different spectral characteristics. The ultraviolet/visible spectra of these compounds are of great importance because they provide valuable information about their structure and content in products [Sant'Anna *et al.*, 2013; Wrolstad & Smith, 2017]. Revealing the relationship between the content of color compounds and their spectral properties in foods by using low-cost and fast techniques is essential to make faster decisions in food processing and preservation [Pathare *et al*., 2013]. In this context, many studies have been conducted based on different foods [Alighourchi & Barzegar, 2009; Gonçalves *et al.,* 2007; Güneşer, 2016; Humphries *et al.,* 2004; Su *et al.,* 2016].

TABLE 4. Multiple linear regression models for pH, betalain content, and color parameters of the yogurts stored at different temperatures.

Temperature (°C)	Multiple regression equation	R^2	*P value*
4	Betalain (mg/kg) = 7.88 L^* + 88.23 a^* + 39.40 b^* + 72.50 pH – 1329	97.55	0.020
10	Betalain (mg/kg) = 3.5 L^* + 85.30 a^* + 41.50 b^* – 7.4 pH – 581	98.59	0.000
20	Betalain (mg/kg) = 6.10 L^* + 39 a^* – 12 b^* – 168 pH + 352	72.09	0.177

[a]Numbers in parentheses are *P* values for regression analysis. L^*: lightness, a^*: redness, b^*: yellowness.

The relationship between the content of betalains, color parameters, and pH of the stored yogurts was explored by multiple linear regression and correlation analyses in the present study. Significant fit of regression models was found for the storage temperatures of 4°C and 10°C with high determination coefficients (R^2 = 97.55 and 98.59) (Table 4). This indicates that the developed models have a reasonable predictive power to calculate the betalain content of the yogurts stored at 4–10°C. The relative weight of the redness (a^*) as a predictor variable had the highest value, followed by yellowness (b^*) in the models. Furthermore, a positive correlation was determined between the content of betalains, a^* value, and pH value, whereas L^* and b^* values showed a negative correlation with betalain content (Table 5).

These findings show that the effective assessment of betalain content in yogurt can be achieved by studying the color values of L^*, a^*, b^*, and pH instead of time-consuming chromatographic analysis. Similarly, in a previous study [Güneşer, 2016], a negative correlation was found for L^* values and betalain contents in milk with a beetroot colorant heated at 70–90°C. Moreover, a significant positive correlation was observed between chroma values and betalain contents. Liaotrakoon *et al.* [2013] showed that a^* and b^* values could be used as criteria to determine the quality of white and red-flesh dragon fruit purees and also that the betalain content of red-flesh dragon fruit purees could be estimated by using the total color change with a high prediction (R^2=0.94). In another study by Gonçalves *et al.* [2007], the total anthocyanin content of four different sweet cherry cultivars (Burlat, Saco, Summit, and Van) was reported to negatively correlate with L^*, a^*, b^*, chroma, and hue angle values during storage at 1.5°C and 15°C. In turn, Humphries *et al.* [2004] investigated the relationship between L^*, a^*, b^* values, and lutein and carotene contents in wheat and triticale samples. The positive correlation found between lutein contents and b^* values was

TABLE 5. Coefficients of correlations between pH, betalain content, and color parameters of the stored yogurts.

	Betalain content	L^*	a^*	b^*
L^*	-0.834 (0.001) [a]			
a^*	0.960 (0.001)	0.853 (0.001)		
b^*	-0.935 (0.001)	0.832 (0.001)	-0.990 (0.00)	
pH	0.720 (0.001)	-0.651 (0.001)	0.727 (0.001)	-0.692 (0.001)

[a]Numbers in parentheses are *P* values determined for correlation analysis. L^*: lightness, a^*: redness, b^*: yellowness.

stronger for durum wheat than that determined for other wheat varieties. In contrast, it was found that carotene and lutein contents were weakly correlated with L^* and a^* values in wheat and triticale samples. It can generally be concluded that such color parameters as L^*, a^*, and b^* are directly related to pigments present in food items. Thus, the color analysis is a useful tool to determine the content of natural pigments of various food items at various processing stages. Sensory color analyses should also be performed in this respect along with instrumental color and chromatographic analyses.

CONCLUSION

This study evaluated the stability of beetroot betalains in yogurts during storage. The pH values of yogurts containing the beetroot extract decreased during the storage, and the decrease was the highest at 20°C. First-order kinetics were reported for the degradation of betalains in yogurts stored at 4°C, 10°C, and 20°C. Moreover, changes in L^*, a^*, and b^* values followed the zero-order kinetics. Storage temperatures had the least effect on L^* value followed by a^* value of color of the yogurts. It was observed that the developed multiple linear regression models had a reasonable predictive power to calculate the betalain content of yogurt samples stored at 4–20°C. The highest value as a predictor variable was obtained for a^* in the models. Further studies should be performed by considering the matrix effect on natural pigments, color parameters, and sensory color properties. All obtained data can be analyzed further using advanced statistical methods to reveal the relationship between color value and natural pigments in food systems.

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CONFLICT OF INTERESTS

The author declares that there is no conflict of interest.

ORCID ID

O. Guneser https://orcid.org/0000-0002-3927-4469

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