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Influence of calcium fortification on the stability of anthocyanins in strawberry puree

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Abstract: Anthocyanins have received an increased attention not only because of its antioxidant activity; but because fortification of food products by minerals is important due to the lack of some minerals in population. The addition of these minerals can affect the sensorial and nutritional composition of food. The influence of calcium fortification on anthocyanins and colour changes in strawberry puree were assessed by accelerated storage test. The quantification of anthocyanins was performed by high-performance liquid chromatography with diode-array detection (HPLC-DAD) and colour changes were measured spectrophotometrically (CIE L^* – lightness, a^* – redness, b^* – yellowness). The kinetical parameters (velocity constants and activation energies) were calculated. The activation energies of degradation of anthocyanins were calculated as pelargonidin-3-glucoside (26.24 ± 0.57, 21.18 ± 1.07, and 24.53 ± 1.33 kJ·mol^{-1}), cyanidin-3-glucoside (16.10 ± 0.96, 11.61 ± 0.74, and 13.34 ± 1.72 kJ·mol^{-1}), and pelargonidin-3-rutinoside (8.91 ± 0.17, 7.39 ± 0.98, and 8.23 ± 1.72 kJ·mol^{-1}) of the control sample, calcium carbonate and calcium citrate respectively. The results showed that the addition of calcium salt had a statistically significant ($P \leq 0.05$) effect on the degradation of anthocyanins.

Keywords: calcium carbonate; calcium citrate; degradation; pelargonidin-3-glucoside; pelargonidin-3-rutinoside; cyanidin-3-glucoside

Anthocyanins are phenolic compounds that impart from red to blue colouration. The main anthocyanidins present in fruits are cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin (Goulas et al. 2012). Their concentration is dependent on the cultivar and growth conditions (Aaby et al. 2012). The stability of anthocyanins is influenced by the processing and storage conditions (e.g. temperature and oxygen), presence of ascorbic acid, and pH (Verbeyst et al. 2010; Buvé et al. 2018). Cations such as alu-

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minium (Al^{3+}) can influence the development of the structure of anthocyanin pigments, which are partially responsible for the colour (Schreiber et al. 2010).

Calcium is required for a variety of metabolic functions by the human body (FAO and WHO 2004). Fruit-based foods are easy to digest and offer high nutrient density. Since infants between 6 months and 3 years are limited in their food choices, commercial baby foods serve as an important source of energy, fibre, vitamins, and minerals and establish their taste and eating patterns (Čížková et al. 2009). It is challenging for infants who do not consume dairy products such as milk, cheese and yoghurt, which are rich sources of calcium, to meet the recommended intake of calcium. This is typically 300 to 500 mg per day according to FAO and WHO (2004). Hence, alternate calcium sources are required. Several commercial calcium salts have been used for calcium enrichment, e.g. calcium carbonate, calcium chloride, calcium phosphate, calcium lactate, and calcium gluconate (Orrego et al. 2014). Again, Vyas and Tong (2004) also investigated the impact of calcium fortification on the heat stability of skim milk powder using calcium carbonate, calcium phosphate, calcium lactate, and calcium citrate.

Humans absorb up to 30% of the calcium in food, which varies with the food consumed (Weaver et al. 2002). The effect of calcium fortification on anthocyanins has received little attention; therefore, there is a need for this investigation.

The hypothetical question is: Does calcium fortification affect the stability of anthocyanin? The aim of this study was to determine the degradation kinetics and anthocyanin stability during storage.

MATERIAL AND METHODS

Ripe strawberries were purchased from Berry servis (Czech Republic). Solvents for high-performance liquid chromatography (HPLC) analysis (HPLC grade Acetonitrile > 99.9%, formic acid 98%) were obtained from Merck (Germany). Calcium citrate and calcium carbonate (98%) were obtained from Lachema (Czech Republic). Anthocyanin standard (pelargonidin 3-glucoside > 97%) was obtained from Polyphenols (Norway).

Model sample preparation. Fresh strawberries were washed and dipped in a water bath at 90 °C for 2 min. They were then shredded into a puree using a laboratory knife mill (GRINDOMIX GM 200) for 60 s at a speed of 3 000 min^{-1}. To meet a 500 mg per day three batches (1 000 g for each treatment) of the puree were fortified with 3.42 and 5.68 g [in 10 mL of distilled water, resulting in a Ca^{2+} concentration of 1.37 g·$(10\ mL)^{-1}$] of calcium carbonate and calcium citrate respectively, and thoroughly mixed using the laboratory knife mill; the third batch as a control sample to meet the recommended dietary allowance (daily) in infants. From atomic absorption spectrophotometric analysis, the fresh puree had a calcium content of 343.64 ± 0.57 mg·kg^{-1}. The pH of the two batches were adjusted to exactly 3.45 using citric acid (pH 4.5, 0.1 M). The homogenised puree was transferred into glass jars (30 mL) and covered with screw caps and coded. Samples were pasteurised in an automatic domo pot (Adler 4496; avXperten, Denmark) at 80 °C for 5 min.

Storage test. The jars were covered with aluminium foil, and stored at temperatures 20, 30, and 40 °C in a thermostat (SBS-LI-65; Steinberg, Denmark) without exposure to light. For each treatment, 2 jars were randomly sampled for measurement of the parameters.

pH. pH was determined according to the Association of Official Analytical Chemists method 981.12 (AOAC 1999).

Anthocyanin extraction and high-performance liquid chromatography analysis. Anthocyanin before HPLC analysis was extracted based on the International Federation of Fruit Juice Producers method (IFU 71; 1998). 10 g of the puree was weighed into a 50 mL centrifuge tube and diluted with 25 mL of distilled water. Thorough mixing was followed by centrifuging for 10 min at 4 500 × g (Centrifuge 5430; Eppendorf, Germany). Finally, the supernatant was filtered through syringe filters (PTFE 0.45 µm) under protection against light before HPLC analysis. Each sample was extracted three times.

The pelargonidin-3-glucoside standard was used for quantification, from which a calibration batch of samples with concentrations 1, 5, 25, 50, and 100 mg·kg^{-1} was prepared, and the anthocyanin content was expressed as the concentration of pelargonidin-3-glucoside (mg·kg^{-1} of the fresh weight). Total anthocyanin in model samples were calculated as the sum of individual anthocyanin expressed as mg·kg^{-1}. High-performance liquid chromatography with diode-array detection (HPLC/DAD; 1260 Infinity; Agilent Technologies, USA) method condition was column C18; column temperature 40 °C; flow rate 1 mL·min^{-1}; injection volume 10 µL; detection 518 nm; analysis time 46 min: mobile phase A (900 mL distilled

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water, 100 mL formic acid) and phase B (400 mL distilled water, 100 mL formic acid, 500 mL acetonitrile). Since the response factor of cyanidin-3-glucoside and pelargonidin-3-rutinoside is similar to pelargonidin 3-glucoside as per IFU 71 (1998) method, quantification of anthocyanins was done according to the retention times.

Colour measurement. The colour was determined as the CIELAB coordinates using a (CM-5; Konica Minolta, Japan) table spectrophotometer (L^* – lightness, a^* – redness, b^* – yellowness).

Determination of calcium content. Samples (1 g of each treatment) were burnt at 550 °C in a Nabertherm P 320 muffle furnace (Nabertherm GmbH, Germany) for 48 h. 3 mL of 1 M hydrochloric acid (HCl) was added to the dry ash for digestion in a 100 mL volumetric flask. In a 10 mL volumetric flask, 2 mL of the digested sample was added to 1 mL of caesium chloride (CsCl). After dilution, calcium was measured using the Atomic Absorption Spectrophotometric (240FS AAS; Agilent, USA) with the AOAC method 984.27 (1999).

Data processing. A first-order reaction model has been applied for describing the degradation of anthocyanin (Verbeyst et al. 2011; Zhao et al. 2012). The model is expressed as:

$$\ln\frac{C}{C_0} = -kt \quad (1)$$

The dependence of the degradation rate constant on calcium addition was determined by applying the Arrhenius equation:

$$K_T = K_0 e^{-\frac{E_a}{RT}} \quad (2)$$

where: C – anthocyanin content after t minutes of heating at a desired temperature; C_0 – initial anthocyanin content; k – rate constant (min^{-1}); K_T – rate constant at specified temperature; K_0 – pre-exponential factor (min^{-1}); E_a – activation energy ($kJ \cdot mol^{-1}$); R – universal gas constant (8.314 $J \cdot K^{-1} \cdot mol^{-1}$); T – absolute temperature (K).

Activation energies were calculated by plotting ln (k_T) against the reciprocal of the absolute temperature (1/T), where the slope of the linear graph is equivalent to $-E_a/RT$.

Statistical analysis. Results are presented as mean values ± standard deviation. Means comparisons were analysed using a Student's t-test. All statistical analyses were performed using Stata/IC software (version 14) and MS Excel (version 2023). All the measurements of calcium content and anthocyanin content were performed in duplicate. The measurement of colour was performed in six replicates.

RESULTS AND DISCUSSION

The addition of 3.42 g of calcium carbonate and 5.68 g of calcium citrate yielded 1 680.46 ± 1.07 and 1 699.00 ± 1.33 $mg \cdot kg^{-1}$ calcium cation (Ca^{2+}) respectively, and 343.64 ± 0.57 $mg \cdot kg^{-1}$ for the control sample.

Strawberry possesses high antioxidants that are positively correlated with the content of polyphenolic compounds and anthocyanins (Wang and Lin 2000). The key issue often found during the processing and storage of strawberry products is the loss of red colour. Calcium fortification largely had an impact on the pH of the model sample. pH < 2.0 favours a bright red colour, while a pH > 4.0 triggers a blue purple colour (Castañeda-Ovando et al. 2009). pH of fortified samples increased from 3.45 to 3.75 and 3.84 in calcium citrate and calcium carbonate respectively. Therefore, there was a need to adjust the pH to 3.45.

Impact of calcium salt on total anthocyanin contents. The main anthocyanins present in strawberries is pelargonidin-3-glucoside, and in smaller portions are cyanidin-3-glucoside and pelargonidin-3-rutinoside (Jakobek et al. 2007). Calcium fortification's effect on anthocyanins' stability is shown in Table 1.

Some authors determined the effect of cations subjected to encapsulation and the stability of anthocyanins as indicators (Ratanapoompinyo et al. 2017; Li 2019). Li (2019) concluded that calcium ions stabilise anthocyanins (analysis carried out in 30 min). Again, it was reported that ferritin nanocarrier enhanced the stability of anthocyanins. The findings explored a novel approach to enhance the stability of anthocyanin. This research suggested a promising method for enhancing the stability of anthocyanin, which could have implications for food preservation and nutritional supplementation (Huang et al. 2023).

However, no work has been done to evaluate the impact of calcium fortification on the stability of anthocyanin, which the present study measured.

Calcium carbonate and calcium citrate reduced the stability of anthocyanins, indicated by the increase

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Table 1. Concentrations of anthocyanins

Temperature (°C)	Anthocyanin	Model sample	Anthocyanin concentration (mg·kg^{-1} of fresh weight) at different storage period (days)									
			0	**1**	**7**	**14**	**22**	**30**	**36**			
		CS	236.79 ± 2.31	234.39 ± 0.99	129.14 ± 2.17	100.33 ± 1.92	72.85 ± 0.88	48.86 ± 1.54	24.67 ± 1.25			
	Pgd-3-glu	CC	218.55 ± 1.37^{b}	217.35 ± 0.82^{a}	120.50 ± 1.95^{c}	54.10 ± 0.40^{a}	41.78 ± 1.51^{a}	30.67 ± 0.30^{a}	15.76 ± 0.76^{b}			
		CT	223.38 ± 1.27^{b}	218.66 ± 2.21^{b}	113.01 ± 1.49^{b}	63.88 ± 1.07^{a}	49.17 ± 1.87^{a}	34.51 ± 0.28^{a}	19.46 ± 0.53^{b}			
		CS	33.04 ± 0.58	32.22 ± 1.60	3.27 ± 0.11	3.25 ± 0.01	3.21 ± 0.07	3.13 ± 0.01	3.02 ± 0.04			
20	Cyd-3-glu	CC	29.68 ± 0.24^{b}	28.11 ± 2.26^{b}	3.24 ± 0.06^{b}	3.21 ± 0.05^{a}	3.17 ± 0.11^{a}	3.09 ± 0.03^{a}	2.94 ± 0.23^{b}			
		CT	31.16 ± 1.07^{c}	31.24 ± 0.90^{b}	3.27 ± 0.00^{b}	3.22 ± 0.09^{a}	3.12 ± 0.89^{a}	3.04 ± 0.27^{a}	3.01 ± 0.00			
		CS	19.53 ± 0.61	18.98 ± 0.93	14.64 ± 0.25	12.51 ± 0.13	10.75 ± 0.14	3.28 ± 0.01	3.15 ± 0.00			
	Pgd-3-rut	CC	18.44 ± 0.43^{b}	15.41 ± 0.33^{a}	11.10 ± 0.10^{c}	7.68 ± 0.07^{a}	6.85 ± 0.03^{a}	3.29 ± 0.03^{a}	2.99 ± 0.23^{b}			
		CT	18.63 ± 0.02^{b}	18.49 ± 0.38^{b}	9.55 ± 0.16^{a}	8.90 ± 0.15^{b}	7.39 ± 0.23^{a}	3.44 ± 0.27^{a}	3.23 ± 0.01^{a}			
		CS	236.79 ± 2.31	223.56 ± 1.27	80.43 ± 0.41	43.05 ± 0.83	35.60 ± 1.31	13.62 ± 0.77	6.35 ± 0.87			
	Pgd-3-glu	CC	218.55 ± 1.37^{b}	201.43 ± 1.09^{a}	48.79 ± 0.41^{a}	27.75 ± 0.59^{a}	17.16 ± 1.44^{a}	12.77 ± 1.76	4.57 ± 0.01^{c}			
		CT	223.38 ± 1.27^{b}	199.13 ± 2.65^{a}	48.51 ± 0.31^{a}	29.94 ± 0.61^{a}	19.76 ± 0.13^{a}	13.61 ± 0.52	5.36 ± 0.00			
		CS	33.04 ± 0.58	29.14 ± 0.15	3.27 ± 0.00	3.23 ± 0.03	3.19 ± 0.44	3.05 ± 1.61	2.91 ± 0.50			
30	Cyd-3-glu	CC	29.68 ± 0.24^{b}	25.87 ± 2.14^{a}	3.23 ± 0.00^{c}	3.19 ± 0.08^{a}	3.13 ± 0.12^{a}	3.06 ± 0.11^{a}	2.96 ± 0.55^{b}			
		CT	31.16 ± 1.07^{b}	25.22 ± 2.32^{b}	3.26 ± 0.00^{c}	3.24 ± 0.00	3.14 ± 0.46^{b}	3.01 ± 0.05^{a}	2.92 ± 1.15^{b}			
		CS	19.53 ± 0.61	18.78 ± 0.44	10.67 ± 0.45	8.88 ± 0.04	6.34 ± 0.88	3.30 ± 0.00	3.03 ± 0.06			
	Pgd-3-rut	CC	18.44 ± 0.43^{b}	14.96 ± 1.34^{a}	7.35 ± 0.06^{a}	5.86 ± 0.20^{a}	5.36 ± 0.00^{a}	3.24 ± 0.02^{a}	2.94 ± 0.00			
		CT	18.63 ± 0.02^{b}	18.57 ± 2.07^{a}	7.23 ± 0.06^{a}	6.08 ± 0.01^{a}	4.84 ± 0.46^{a}	3.24 ± 0.09^{a}	3.01 ± 0.04^{a}			
			0	**1**	**3**	**7**	**10**	**14**	**17**	**22**	**25**	**30**
		CS	236.79 ± 2.31	175.32 ± 2.69	96.78 ± 1.07	35.71 ± 0.58	31.02 ± 0.60	21.94 ± 0.40	16.89 ± 0.69	12.18 ± 0.63	9.98 ± 0.71	5.74 ±0.28
	Pgd-3-glu	CC	218.55 ± 1.37^{b}	131.21 ± 0.03^{a}	64.25 ± 0.54^{a}	22.38 ± 0.95^{a}	18.27 ± 0.16^{a}	10.47 ± 0.37^{a}	9.68 ± 0.24^{a}	7.30 ± 0.59^{b}	6.87 ± 0.01^{b}	3.48 ±0.01^{a}
		CT	223.38 ± 1.27^{b}	156.88 ± 0.28^{b}	65.08 ± 1.76^{a}	24.40 ± 0.32^{a}	21.05 ± 1.52^{b}	12.90 ± 0.35^{a}	11.60 ± 0.00^{a}	7.51 ± 0.36^{b}	7.86 ± 0.30^{c}	3.51 ±0.19^{b}
		CS	33.04 ± 0.58	22.04 ± 2.14	10.34 ± 2.06	3.95 ± 0.01	3.25 ± 0.01	3.11 ± 0.04	3.07 ± 0.09	3.01 ± 0.69	3.00 ± 0.27	2.91 ±0.31
40	Cyd-3-glu	CC	29.68 ± 0.24^{b}	18.76 ± 1.97^{a}	8.45 ± 0.96^{a}	3.22 ± 0.00^{a}	3.20 ± 0.15^{b}	3.17 ± 0.11^{a}	3.12 ± 0.00^{a}	3.02 ± 0.18^{a}	2.89 ± 0.68^{c}	2.73 ±0.34^{b}
		CT	31.16 ± 1.07^{b}	15.93 ± 1.11^{b}	5.74 ± 1.67^{a}	3.20 ± 0.05^{a}	3.16 ± 0.00^{a}	3.14 ± 0.65^{a}	3.09 ±0.43^{a}	3.03 ± 0.58^{b}	2.98 ± 0.41^{b}	2.86 ±0.15^{a}
		CS	19.53 ± 0.61	16.12 ± 0.04	11.04 ± 0.22	6.14 ± 0.29	6.24 ± 0.19	5.35 ± 0.05	4.91 ±0.01	4.34 ± 0.28	3.24 ± 0.00	3.09 ±0.07
	Pgd-3-rut	CC	18.44 ± 0.43^{a}	12.47 ± 0.26^{b}	8.26 ± 0.54^{a}	5.60 ± 0.16^{a}	4.86 ± 0.32^{a}	4.43 ± 0.02	4.04 ±0.27^{b}	4.06 ± 0.02	3.24 ± 0.09^{a}	3.03 ±0.04^{a}
		CT	18.63 ± 0.02	14.48 ± 0.01^{b}	8.53 ± 0.29^{a}	5.71 ± 0.35^{a}	5.18 ± 0.35^{b}	4.41 ± 0.02	4.11 ±0.06	3.88 ± 0.01	3.09 ± 0.00	2.99 ± 0.05^{b}

$^{a, b, c}$ Different letters represent significant differences by Student's *t*-test at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively; CS – control sample; CC – calcium carbonate; CT – calcium citrate; Pgd-3-glu – pelargonidin-3-glucoside; Cyd-3-glu – cyanidin-3-glucoside; Pgd-3-rut – pelargonidin-3-rutinoside

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in degradation kinetics constants as compared to the control sample (Table 1). During accelerated storage, anthocyanin stability was significantly decreased in the model samples. Differences in treatments were obvious during further accelerated storage tests. Model samples with calcium citrate provided better anthocyanin stabilisation as compared to calcium carbonate (Table 1).

Studies show that degradation of anthocyanin follow a first-order reaction. To verify this, in C/C_0 is plotted against time (Figure 1). Our findings were in agreement with the observation of Cabrita et al. (2000), Garzon and Wrolstad (2002). It can be suggested that the temperature dependence of the total anthocyanin in the model samples with calcium are statistically significant (Table 2). The results indicate a rapid degradation at 40 °C which indicates that degradation kinetics was strongly affected by the temperature. Cyanidin-3-glucoside, pelargonidin-3-glucoside and pelargonidin-3-rutinoside concentration decreased by 87, 97, and 92%, respectively. Levels of pelargonidin-3-glucoside, declined with the higher kinetic rate (Table 2).

A high activation energy indicates a higher sensitivity of the reaction rate to temperature. Results are lower than results reported in the literature for anthocyanin degradation in other red fruit over the range of 21 to 100 °C (Harbourne et al. 2008; Sinela et al. 2017).

Colour change. Colour is affected by pH (Fossen et al. 1988), and the results of colour measurement showed that a^* and L^* values significantly decreased after the pH was adjusted to 3.45 as reported by Espin et al. (2000) and Buvé et al. (2018). Since red colour is predominantly induced by the anthocyanins, the change in red colour could be resulted from anthocyanin degradation.

Degradation rate constants increased with higher temperatures, indicating that the colour became darker at higher temperatures (Table 3). It can be suggested that the colour changed with a degradation in anthocyanin concentration, with E_a values of colour recorded as 8.37 ± 0.60, 4.77 ± 0.16, and 5.41 ± 0.31 kJ·mol^{-1} for the control sample, calcium carbonate and calcium citrate respectively. According to the comparison of the degradation kinetic parameters of anthocyanin and colour, E_a anthocyanin degradation (Figure 2) was higher than that of colour decrease, which meant that the temperature dependence of anthocyanin degrada-

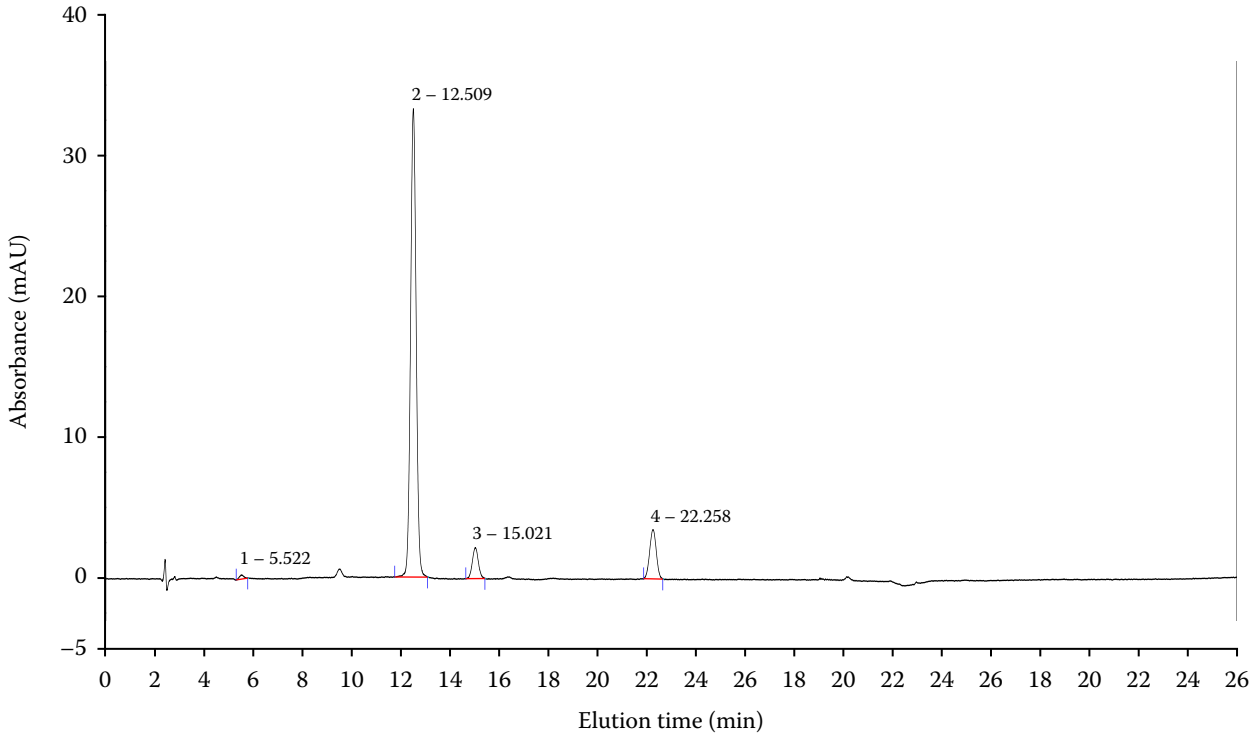

Figure 1. Anthocyanins chromatogram

Peak 1 – unknown sample; peak 2 – pelargonidin-3-glucoside; peak 3 – cyanidin-3-glucoside; peak 4 – pelargonidin-3-rutinoside; the individual anthocyanins were identified according to retention times in the International Federation of Fruit Juice Producers method (IFU 71; 1998)

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Table 2. Kinetic parameters of anthocyanin degradation

Anthocyanin	Calcium salt	Temperature (°C)	Kinetic equation of 1st order	k (day^{-1})	R^2	E_a (kJ·mol^{-1})
Pgd-3-glu	CS	20	$y = 231.38e^{-0.06x}$	−0.06 ± 0.00	0.97	26.24 ± 0.57
		30	$y = 210.09e^{-0.09x}$	−0.09 ± 0.01	0.97	
		40	$y = 139.80e^{-0.11x}$	−0.11 ± 0.01	0.93	
	CC	20	$y = 203.01e^{-0.07x}$	−0.07 ± 0.01	0.97	21.18 ± 1.07[b]
		30	$y = 161.24e^{-0.10x}$	−0.10 ± 0.01	0.94	
		40	$y = 99.38e^{-0.12x}$	−0.12 ± 0.02	0.88	
	CT	20	$y = 202.85e^{-0.07x}$	−0.06 ± 0.00	0.97	24.53 ± 1.33[b]
		30	$y = 161.38e^{-0.09x}$	−0.09 ± 0.01	0.93	
		40	$y = 110.5e^{-0.12x}$	−0.12 ± 0.02	0.89	
Cyd-3-glu	CS	20	$y = 29.02e^{-0.08x}$	−0.06 ± 0.02	0.84	16.10 ± 0.96
		30	$y = 29.10e^{-0.07x}$	−0.07 ± 0.02	0.80	
		40	$y = 27.91e^{-0.10x}$	−0.08 ± 0.02	0.81	
	CC	20	$y = 26.35e^{-0.07x}$	−0.08 ± 0.02	0.87	11.61 ± 0.74[a]
		30	$y = 27.47e^{-0.08x}$	−0.10 ± 0.02	0.82	
		40	$y = 26.35e^{-0.09x}$	−0.12 ± 0.02	0.86	
	CT	20	$y = 27.59e^{-0.08x}$	−0.07 ± 0.02	0.81	13.34 ± 1.72[c]
		30	$y = 26.36e^{-0.064x}$	−0.09 ± 0.02	0.84	
		40	$y = 25.23e^{-0.09x}$	−0.09 ± 0.02	0.81	
Pgd-3-rut	CS	20	$y = 21.83e^{-0.05x}$	−0.06 ± 0.01	0.92	8.91 ± 0.17
		30	$y = 19.16e^{-0.05x}$	−0.06 ± 0.00	0.96	
		40	$y = 16.79e^{-0.04x}$	−0.06 ± 0.01	0.97	
	CC	20	$y = 18.44e^{-0.05x}$	−0.05 ± 0.01	0.97	7.39 ± 0.98[a]
		30	$y = 16.54e^{-0.05x}$	−0.05 ± 0.01	0.94	
		40	$y = 14.12e^{-0.04x}$	−0.05 ± 0.01	0.89	
	CT	20	$y = 13.55e^{-0.05x}$	−0.04 ± 0.00	0.84	8.23 ± 1.72[a]
		30	$y = 12.04e^{-0.05x}$	−0.05 ± 0.01	0.80	
		40	$y = 10.83e^{-0.04x}$	−0.05 ± 0.01	0.74	

[a, b, c] Different letters represent significant differences by Student's t-test at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively; k – rate constant; E_a – activation energy; Pgd-3-glu – pelargonidin-3-glucoside; Cyd-3-glu – cyanidin-3-glucoside; Pgd-3-rut – pelargonidin-3-rutinoside; CS – control sample; CC – calcium carbonate; CT – calcium citrate

tion was greater. This might be due to the hyperchromic effect caused by metallic ions. The increase of ionic strength in aqueous food leads to the formation of ion pairs by highly unstable anthocyanidins, contributing significantly to its stability and the decrease in lightness (Figueiredo and Pina 1994: Reyes and Cisneros Zevallos 2007).

Colour change could be used for the estimation of the shelf life of strawberry products. The calculated kinetic equations can be used for extrapolation of shelf life at model storage temperatures (Prchalová et al. 2016). At a statistical significance level ($P \leq 0.05$) anthocyanins were affected by calcium salt.

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Table 3. Effect of calcium salt on colour

Temperature (°C)	Colour characteristics	Model sample	Effect of calcium salt on colour at different storage period (days)									
			0	**1**	**7**	**14**	**22**	**30**	**36**			
20	L^*	CS	34.71 ± 0.02	34.33 ± 0.08	34.63 ± 0.06	35.51 ± 0.08	35.90 ± 0.03	37.70 ± 0.04	38.46 ± 0.06			
		CC	33.39 ± 0.02^b	34.94 ± 0.06^b	34.32 ± 0.03^c	37.43 ± 0.02^a	34.70 ± 0.06^b	37.24 ± 0.06^a	38.01 ± 0.13^c			
		CT	36.22 ± 0.08^b	37.01 ± 0.09^b	36.27 ± 0.11^b	37.36 ± 0.18^a	37.11 ± 0.09^b	37.78 ± 0.11^c	38.21 ± 0.05^c			
	a^*	CS	14.69 ± 0.06	16.28 ± 0.10	13.39 ± 0.12	12.22 ± 0.06	9.15 ± 0.11	7.87 ± 0.06	6.56 ± 0.06			
		CC	16.69 ± 0.01^a	13.61 ± 0.05^b	12.10 ± 0.07^c	8.27 ± 0.14^a	6.00 ± 0.05^c	6.19 ± 0.13^a	5.90 ± 0.04^b			
		CT	16.32 ± 0.02^a	15.84 ± 0.06^c	11.22 ± 0.08^a	8.15 ± 0.06^c	5.98 ± 0.01^b	5.67 ± 0.19^a	4.96 ± 0.12^b			
30	L^*	CS	34.71 ± 0.13	36.24 ± 0.05	36.83 ± 0.06	37.34 ± 0.26	36.10 ± 0.20	36.74 ± 0.02	36.99 ± 0.019			
		CC	33.93 ± 0.06^a	35.73 ± 0.19^c	35.59 ± 0.09^c	35.91 ± 0.06^a	35.54 ± 0.06^b	36.05 ± 0.13^c	36.45 ± 0.08^b			
		CT	34.22 ± 0.11^b	35.63 ± 0.11^a	37.00 ± 0.15^b	36.79 ± 0.18^a	36.13 ± 0.17^a	35.87 ± 0.03	36.67 ± 0.13^c			
	a^*	CS	14.69 ± 0.01	14.93 ± 0.02	11.49 ± 0.02	7.92 ± 0.01	6.24 ± 0.05	6.06 ± 0.02	5.35 ± 0.02			
		CC	16.69 ± 0.06^a	14.59 ± 0.12^c	6.12 ± 0.06^c	5.48 ± 0.02^b	4.73 ± 0.04^a	4.85 ± 0.03^b	4.01 ± 0.06^b			
		CT	16.32 ± 0.12^a	14.35 ± 0.08^c	6.44 ± 0.05^a	5.54 ± 0.09^a	4.95 ± 0.10^a	4.54 ± 0.08^b	3.91 ± 0.07^b			
			0	**1**	**3**	**7**	**10**	**14**	**17**	**22**	**25**	**30**
40	L^*	CS	34.71 ± 0.02	35.55 ± 0.01	35.01 ± 0.11	34.98 ± 0.02	34.23 ± 0.15	33.96 ± 0.02	33.75 ± 0.24	33.21 ± 0.09	33.07 ± 0.03	32.47 ± 0.08
		CC	33.93 ± 0.05^b	34.21 ± 0.09^a	33.89 ± 0.02^b	34.11 ± 0.06^a	33.92 ± 0.12^a	33.04 ± 0.15^b	33.36 ± 0.21^b	31.77 ± 0.14	33.44 ± 0.02^c	32.20 ± 0.12^b
		CT	34.22 ± 0.08^b	35.44 ± 0.11^a	35.02 ± 0.07^a	33.69 ± 0.03^b	33.99 ± 0.19^b	33.61 ± 0.14^b	33.02 ± 0.02^a	32.80 ± 0.02^a	33.03 ± 0.11^c	32.68 ± 0.22^b
	a^*	CS	14.69 ± 0.02	15.17 ± 0.19	8.44 ± 0.03	5.94 ± 0.16	5.53 ± 0.23	4.99 ± 0.06	4.26 ± 0.04	3.99 ± 0.11	3.83 ± 0.05	3.75 ± 0.06
		CC	16.69 ± 0.03^c	11.54 ± 0.04^c	7.69 ± 0.05^a	4.48 ± 0.02^a	4.18 ± 0.26^b	4.28 ± 0.02^b	4.15 ± 0.02^c	4.03 ± 0.19^c	3.86 ± 0.07^b	4.09 ± 0.02^b
		CT	16.32 ± 0.25^c	11.04 ± 0.14	7.73 ± 0.13^c	4.27 ± 0.13^b	4.39 ± 0.02^b	4.27 ± 0.15^b	3.95 ± 0.05^b	3.36 ± 0.03^a	3.26 ± 0.01^a	3.44 ± 0.09^c

$^{a, b, c}$ Different letters represent significant differences by Student's t-test at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively; L^* – lightness; a^* – redness; CS – control sample; CC – calcium carbonate; CT – calcium citrate

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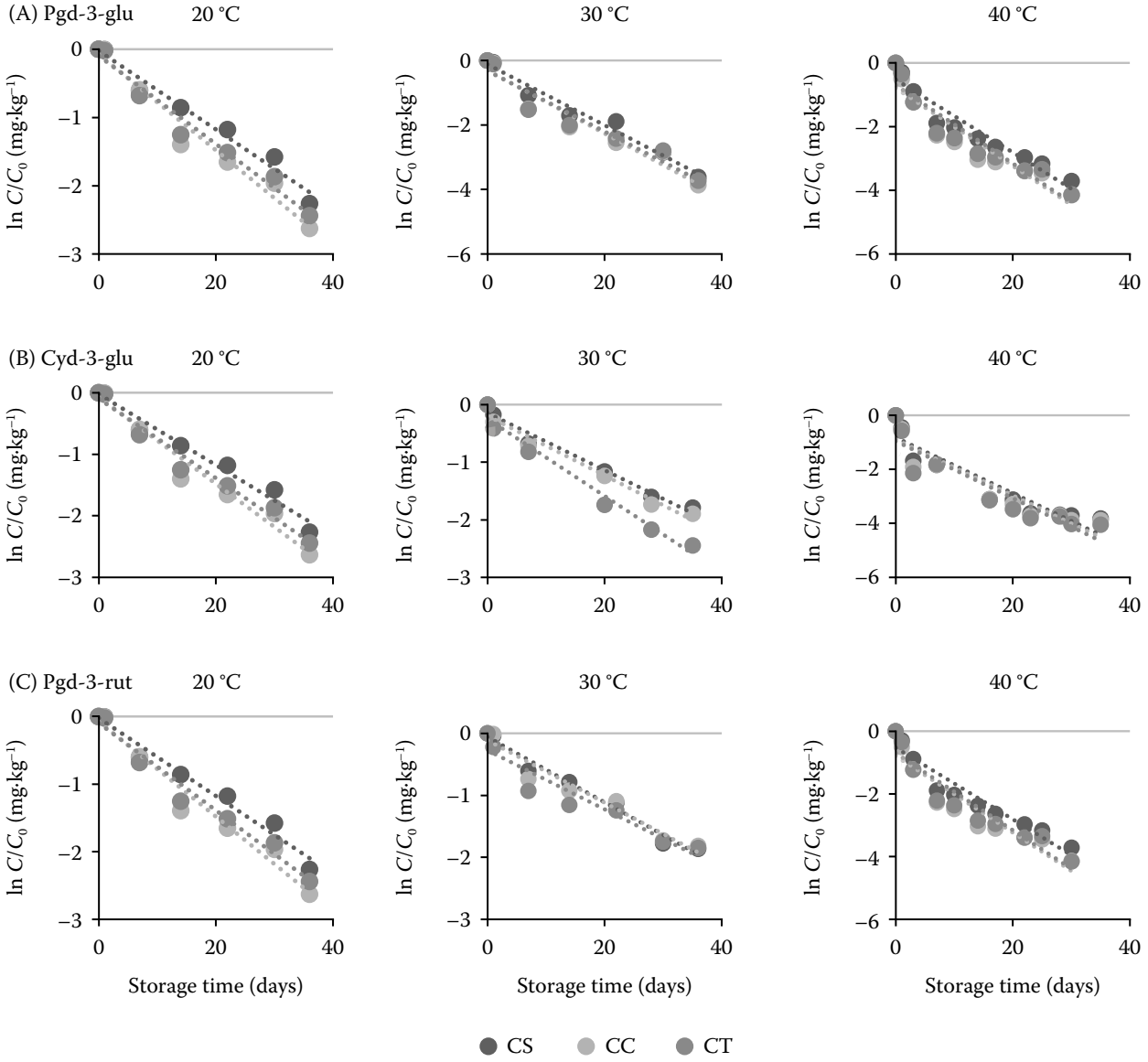

Figure 2. Degradation of anthocyanins during storage: (A) Pgd-3-glu at 20, 30, and 40 °C, (B) Cyd-3-glu at 20, 30, and 40 °C, and (C) Pgd-3-rut at 20, 30, and 40 °C

Pgd-3-glu – pelargonidin-3-glucoside; Cyd-3-glu – cyanidin-3-glucoside; Pgd-3-rut – pelargonidin-3-rutinoside; C – anthocyanin content after t minutes of heating at a desired temperature; C_0 – initial anthocyanin content; CS – control sample; CC – calcium carbonate; CT – calcium citrate

CONCLUSION

From the results, it can be concluded that the stability of anthocyanins in calcium-fortified strawberry puree subjected to accelerated storage treatment was negatively affected. The degradation of anthocyanins agreed with the changes in colour during storage. The mechanism of degradation reactions was relatively complex and further investigation should be conducted to elucidate the roles of metal ion fortification in the degradation of anthocyanins. The scientific results provide researchers and food manufacturers with to understand the anthocyanin behaviour under thermal processing and calcium fortification to apply the most suitable method for maximising their retention and preserving their biological value.

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