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Effect of Thermal Processing on Anthocyanin Degradation in Two Bilberry Jam Formulations

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***Abstract**: Fresh bilberries were processed into sugar and sugar-low jams at high temperatures (90, 95, 100 and 105 °C) in order to follow degradation of total and individual anthocyanins. The greatest retention of all examined compounds was observed in sugar-low jam prepared at 90 °C for 5 minutes, while the greatest loss was detected in sugar jam prepared at 105 °C for 30 min. Cyanidin-3-galactoside and cyanidin-3-glucoside were found to be the most stable, while delphinidin-3-arabinoside and petunidin-3-arabinoside were the least stable.*

***Keywords**: anthocyanins, antioxidant activity, bilberry, degradation kinetics*

1.Introduction

Anthocyanins are water-soluble pigments which are responsible for blue, violet, pink and red colors of fruits of some plants [1]. They are found in strawberries [2], bilberries, blackcurrants and cowberries [3], raspberries and blackberries [4, 5]. Anthocyanins represent potent natural antioxidants with cardioprotective, anti-inflammatory and anticancer properties [5]. Bilberries (*Vaccinium myrtillus*) are one of the richest natural sources of anthocyanins [6] where they comprise up to 90% of total phenolic compounds [3]. Antioxidant activity of bilberries has been evaluated in several studies [7-9] and has been given in review works of Heinonen [10] and Szajdek and Borowska [11]. Therefore, bilberries are considered as functional food, with a beneficial effect on human health [11]. However, because of the seasonal character of bilberry fruits, it is important to determinate whether thermally processed forms, such as jams, could also represent a good source of nutrients.

Thermal degradation kinetics of anthocyanins follows first-order kinetics, where the rate constant increases with an increase in temperature [12]. Several published studies agree with previously mentioned [13-15].

According to de Moura et al. [15], the low-sugar blackberry jam prepared at 95°C, can be considered a source of anthocyanin compounds even after six months of storage at an average temperature of 10°C, since 19% loss of total anthocyanin content has been reported. García-Viguera et al. [16] reported losses in total anthocyanin content up to 40% (comparing to fresh fruit) during red raspberry jam manufacturing, with the jam being heated at 92°C. In a study of Howard et al. [17], retention of 79% of total anthocyanins in sugar and sugar-free blueberry jams, which were heated until reached a full boil and held for 1 min on 103-105 °C, has been observed.

But according to our knowledge, the influence of high temperatures, which are often applied during bilberry jam preparation, on the content of total and individual anthocyanins has not been researched comparatively. Therefore, the objective of this study was to examine the influence of high temperatures and sugar on total and individual anthocyanin content, as well as an examination of anthocyanin degradation kinetic. Also, total phenolic and flavonoid contents and antioxidant activity by DPPH assay were determined in sugar and sugar-low bilberry jams

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2.Materials and methods

Trolox was bought from Acros Organics (Morris Plains, New Jersey, USA). Malvidin-3-*O*-glucoside chloride, delphinidin-3-*O*-glucoside chloride, gallic acid, (+)-catechin, DPPH (HPLC grade), saccharose and glucose were purchased from Sigma Aldrich (Steinheim, Germany). Cyanidin-3-*O*-glucoside chloride (HPLC grade) was from ChromaDex (Irvine, CA, USA). Folin Ciocalteu's phenol reagent, sodium hydroxide, sodium acetate, sodium nitrite, sodium carbonate, potassium chloride, aluminum chloride hexahydrate, and hydrochloric acid were purchased from Merck® (Darmstadt, Germany). Methanol (HPLC grade) and ethanol (96% by vol.) were purchased from J.T. Baker (Deventer, The Netherlands). For preparation of all samples and standards purified water (18 MΩcm) was used (prepared by a MicroMed purification system (TKA Wasseraufbereitungssysteme GmbH, Niederelbert, Germany).

Instruments

The spectrophotometer Agilent 8453 UV/Vis (Agilent Technologies, Santa Clara, USA) was used for absorbance measurements. The spectra were recorded by using optical cuvettes of 1 cm optical path. The Hanna instrument pH-meter (Hanna Instruments, Smithfield, Rhode Island, USA) equipped with a glass electrode was used for the pH measurements. A model 1200 (Agilent Technologies, Santa Clara, USA) was used for HPLC analysis. The separation was performed in C_{18} Zorbax Eclipse XDB-C18 column, 5μm, 4.6×150 mm (Agilent Technologies, Santa Clara, USA).

Samples

Bilberry samples were harvested in Southeastern Serbia (Vlasina region). The amount of berries collected for the analysis was about 500 g. Before the analysis, the samples (whole fresh fruits) were stored in the fridge at -18 °C. Frozen bilberry fruits were milled in the blender to obtained puree which was subsequently used for jam preparation. Two formulations of jams (sugar and sugar-low) were prepared according to a slightly modified method described by de Moura et al. [15]. The sugar formulation implied 60% of fruit puree, 30% of saccharose, 9.8% of glucose and 0.2% of commercially available pectin for domestic use. The sugar-low formulation implied 94.2% of fruit puree, 5% of saccharose and 0.8% of commercially available pectin for domestic use.

Heating procedure

The mixtures were heated to temperatures of 90 ºC, 95 ºC, 100 ºC, and 105 ºC and frequently stirred in opened vessels. During the heat treatment, the temperature was registered using laboratory thermometer and stabilized at process temperature (±1ºC). Once isothermal conditions were reached, samples were taken at different heating times: 0, 5, 10, 15, 20 and 30 min. In order to stop further thermal degradation, the samples were immediately immersed into cold water. The analysis was done immediately.

Extraction procedure

The ultrasonic extractions of jam samples were performed at room temperature (25°C) for 15 min three times. Amount of 5.0000 g ± 0.0001 g of each sample was weighed, then was mixed with 5 mL acidified methanol with HCl (1%) and put into the ultrasonic bath and sonicated for 15 min. All the extracts were filtered, and the clear supernatants were collected. Three times repeated extractions were made, the fractions were collected and evaporated to dryness by rotary evaporation under reduced pressure at 40°C. Ultrapure water was added to 10 mL and these solutions were used for further analysis.

HPLC analysis

To identify and determine the individual anthocyanins content Agilent-1200 series HPLC with the UV-Vis photodiode array detector (DAD) was used. The column was thermostated at 25°C. After injecting 5 μL of sample, the separation was performed in an Agilent-Eclipse XDB C-18 4.6×150 mm column. The mobile phase consisted of two solvents, which were used for gradient eluation: 5% formic

acid, aqueous (eluent A) and 80% acetonitrile/5% formic acid/15% purified water (eluent B). The applied elution program was described in detail by Mitić et al. [18]. Identifications of individual compounds were based on the retention times and spectral data with those of the standards or with data (petunidin-3-galactoside, peonidin-3-galactoside, and petunidin-3-arabinoside) reported in the literature [19, 20]. Quantitative determination of individual anthocyanins in samples was calculated using calibration curves. Petunidin-3-galactoside, peonidin-3-galactoside, petunidin-3-arabinoside, cyaniding-3-arabinoside, and cyaniding-3-galactoside were quantified using the calibration curve of cyanidin-3-*O*-glucoside [19]. Delphinidin-3-galactoside and delphinidin-3-arabinoside were quantified using the calibration curve of delphinidin-3-*O*-glukoside. Triplicate measurements were taken, and data were presented as mean ± standard deviation (SD).

Determination of total polyphenols, flavonoids, anthocyanins and antioxidant activity

The content of total polyphenols was determined according to the Folin-Ciocalteu procedure [21-23] using gallic acid as standard and expressing the results as gallic acid equivalents (GAE) per gram of jam samples (mg GAE/g). Content of total flavonoid was measured by the aluminum chloride spectrophotometric method described by Zhishen et al. [24], with catechin as a standard. The results were expressed as a gram of catechin equivalents (CE) per gram of jam samples (mg CE/g). The total anthocyanin content of the acidified methanol extracts was determined using the pH-differential method [25] and expressed as miigrams of cyanidin-3-*O*-glucoside equivalents/g jam (mg cy-3-glu/g). For the DPPH method [26], which is slightly modified, a solution of DPPH (1×10^{-4} mol/L) was prepared in methanol. 5.0 mL of DPPH solution were mixed with 100 µL of jam extract and was filled with methanol to 10 mL. 30 min after the reaction began the discoloration of the DPPH radical was measured at 520 nm. The calibration curve (Trolox equivalent) was plotted as a function of the decrease in absorbance of DPPH radical scavenging activity. The results were given as millimoles of Trolox equivalents (TE) per gram of jam sample (mmol TE/g).

Statistical analysis

All the data are presented as the mean ± standard deviation (SD) for triplicate determinations. Differences in the antioxidant activity and total polyphenols, flavonoids and anthocyanins content samples were tested by the Tukey's test. Statistical analysis was performed using a statistical package running on a computer (Excel Microsoft Office 365). A probability of $p < 0.05$ was considered to be statistically significant [27].

3.Results and discussions

According to obtained results given in Table 1, total polyphenolic (TP), total flavonoid (TF), total anthocyanin (TA) contents and antioxidant activity of jam samples decreased in time and temperature-dependent manners but is more noticeable in sugar than in sugar-low jam at all applied temperatures.

Slight loss in TP, TF, TA, and antioxidant activity was observed after 5 min of heating with retention percentage yielding approximately 75-91, 79-87, 59-74 and 82-90% in sugar jam and 87-97%, 90-97%, 85-95% and 90-94% in sugar-low jam samples at temperatures 105, 100, 95 and 90°C, respectively.

However, significant to moderate loss in TP, TF, TA, and antioxidant activity contents was noticed after 20 min of heating, with retention percentage yielding approximately 30-47, 45-57, 28-57 and 38-62% in sugar jam and 50-70, 57-76, 54-76 and 77-90% in sugar-low jam at temperatures 105, 100, 95 and 90°C, respectively.

The most significant decrease in TP, TF, and TA contents, and antioxidant activity, after 30 m of the thermal treatment at 105 °C, was observed. Retention percentages yielding 26.4, 39.6, 23.9 and 31.6% in sugar jam, and 40.4, 53.6, 50.2 and 62.8% in sugar-low jam, respectively.

Furthermore, strong positive correlations were observed between DPPH antioxidant capacity and total phenols, flavonoids and anthocyanins ($R^2 = 0.9480$; $p<0.0001$; $R^2 = 0.9659$; $p<0.004$; $R^2 = 0.9835$; $p<0.0001$)

HPLC analysis of individual anthocyanins

The results regarding changes in the content of individual anthocyanins in sugar jam and in the sugar-low jam are represented in Table 2 and Table 3. Ten peaks were identified: delphinidin-3-*O*-galactoside, delphinidin-3-*O*-glucoside, cyanidin-3-*O*-galactoside, delphinidin-3-arabinoside, cyanidin-3-*O*- glucoside, petunidin-3-galactoside, cyanidin-3-*O*-arabinoside, peonidin-3-galactoside, petunidin-3-*O*-arabinoside and malvidin-3-galactoside. Glycosides of delphinidin were the most abundant, followed by glycosides of petunidin and cyanidin, while the galactosides of peonidin and malvidin were minor constituents. Preparation of jam of both formulations at 90°C resulted in the greatest retention of all identified individual anthocyanins.

Comparing anthocyanidins which contained galactose in jam samples which were heated at 90°C for 30 min, the highest retention was observed at cyanidin-3-*O*-galactoside (73.1%), followed by peonidin-3-galactoside (68.4%), petunidin-3-galactoside (67.8%), delphinidin-3-*O*-galactoside (51.9%) and malvidin-3-galactoside (41.5%) in sugar jam. The retention of galactosides in sugar-low jam was higher and followed the same order: cyanidin-3-*O*-galactoside (88.0%) > peonidin-3-galactoside (85.2%) > petunidin-3-galactoside (79.5%) > delphinidin-3-*O*-galactoside (78.6%) > malvidin-3-galactoside (70.5%). There were two identified anthocyanidins containing glucose, with cyanidin-3-*O*-glucoside retaining better (80.5%) than delphinidin-3-*O*-glucoside (60.6%) in sugar jam. Also, in sugar-low jam cyanidin-3-*O*-glucoside retained slightly better (85.4%) than delphinidin-3-*O*-glucoside (83.6%). Among three arabinosides, the highest retention was observed at cyanidin-3-*O*-arabinoside, followed by petunidin-3-*O*-arabinoside and delphinidin-3-arabinoside and yielding 62.1%, 60.7% and 57.5% in sugar jam and 72.9%, 72.3% and 69% in sugar-low jam, respectively

Table 1
Total phenolic (TP), total flavonoid (TF), total anthocyanin (TA) contents and antioxidant activity during processing of sugar and sugar-low bilberry jams

Temp (°C)	Time (min)	TP (mg GAE/g)				TF (mg CE/g)			
		Sugar jam	RSD (%)	Low-sugar jam	RSD (%)	Sugar jam	RSD (%)	Low-sugar jam	RSD (%)
		4.02±0.03^{a*}	0.70*	5.29±0.01^{a*}	0.27*	0.348±0.004^{a*}	1.02*	0.399±0.003^{a*}	0.71*
90	5	3.66±0.06^{a}	1.55	5.11±0.02^{a}	0.33	0.302±0.006^{ab}	1.88	0.388±0.001^{a}	0.36
	10	2.85±0.06^{a}	1.98	4.43±0.01^{a}	0.29	0.262±0.004^{b}	1.62	0.355±0.001^{a}	0.40
	15	2.32±0.01^{a}	0.61	4.02±0.04^{a}	1.09	0.227±0.001^{bc}	0.62	0.322±0.001^{a}	0.22
	20	1.89±0.01^{ab}	0.75	3.70±0.03^{a}	0.91	0.200±0.002^{bcd}	1.06	0.304±0.002^{a}	0.70
	30	1.69±0.01^{ab}	0.84	3.28±0.04^{ab}	1.31	0.182±0.003^{bcd}	1.55	0.291±0.002^{a}	0.73
95	5	3.54±0.01^{abc}	0.20	5.00±0.07^{ab}	1.41	0.295±0.001^{bcd}	0.48	0.383±0.002^{a}	0.55
	10	2.52±0.06^{abc}	2.24	4.20±0.04^{ab}	0.92	0.251±0.001^{bcd}	0.56	0.343±0.003^{a}	0.82
	15	1.89±0.01^{abcd}	0.38	3.52±0.03^{ab}	0.96	0.205±0.003^{bcd}	1.38	0.299±0.004^{a}	1.18
	20	1.59±0.01^{abcd}	0.89	3.31±0.04^{ab}	1.13	0.182±0.001^{bcd}	0.78	0.283±0.001^{a}	0.50
	30	1.36±0.07^{abcda}	5.20	3.19±0.03^{ab}	0.82	0.162±0.001^{bcd}	0.87	0.270±0.001^{a}	0.52
100	5	3.32±0.03^{abcda}	0.85	4.77±0.05^{ab}	0.95	0.281±0.001^{bcd}	0.50	0.374±0.003^{a}	0.76
	10	2.26±0.04^{abcda}	1.88	3.82±0.05^{ab}	1.19	0.235±0.001^{bcd}	0.60	0.314±0.001^{a}	0.45
	15	1.60±0.04^{abcda}	2.65	3.09±0.08^{abc}	2.59	0.191±0.003^{bcd}	1.48	0.273±0.001^{a}	0.52
	20	1.30±0.03^{abcda}	2.18	2.95±0.05^{abc}	1.63	0.171±0.001^{bcd}	0.83	0.253±0.003^{a}	1.12
	30	1.21±0.01^{abcda}	1.11	2.24±0.04^{abc}	1.97	0.152±0.002^{bcd}	1.39	0.237±0.001^{a}	0.60
105	5	3.01±0.03^{abcda}	0.94	4.63±0.01^{abc}	0.31	0.275±0.002^{bcd}	0.77	0.360±0.002^{a}	0.59
	10	1.98±0.04^{bcda}	2.14	3.27±0.01^{bc}	0.30	0.217±0.001^{cd}	0.65	0.298±0.004^{ab}	1.18
	15	1.32±0.01^{cda}	0.53	2.78±0.02^{c}	0.69	0.171±0.002^{d}	1.25	0.251±0.001^{ab}	0.56
	20	1.19±0.01^{da}	0.60	2.63±0.02^{c}	0.70	0.156±0.001^{d}	0.91	0.227±0.003^{ab}	1.25
	30	1.06±0.01^{e}	0.60	2.14±0.04^{c}	1.66	0.138±0.002^{d}	1.53	0.214±0.004^{b}	1.66

Table 1 (Continuated)

Temp. (°C)	Time (min)	TA (mg cy-3-gly/g)				DPPH (mmol TE/g)			
		Sugar jam	RSD (%)	Low-sugar jam	RSD (%)	Sugar jam	RSD (%)	Low-sugar jam	RSD (%)
		1.88±0.01^{a*}	0.71*	2.41±0.02^{a*}	0.94*	3.01±0.05*	1.83*	3.55±0.08^{a*}	2.22*
90	5	1.39±0.01^{ab}	0.87	2.28±0.02^{ab}	1.05	2.71±0.03^{a}	1.02	3.35±0.07^{ab}	2.13
	10	1.26±0.01^{ab}	1.07	2.14±0.04^{ab}	2.01	2.38±0.01^{a}	0.59	3.30±0.04^{ab}	1.24
	15	1.16±0.02^{bc}	1.33	1.98±0.02^{ab}	0.93	2.14±0.01^{a}	0.56	3.24±0.06^{ab}	1.90
	20	1.07±0.01^{bc}	0.99	1.83±0.02^{ab}	0.93	1.86±0.04^{ab}	2.05	3.18±0.02^{ab}	0.70
	30	0.93±0.01^{bc}	1.29	1.63±0.05^{abc}	3.13	1.48±0.02^{ab}	1.29	3.08±0.03^{ab}	1.11
95	5	1.33±0.01^{bc}	1.06	2.21±0.06^{abc}	2.59	2.63±0.07^{ab}	2.50	3.32±0.08^{ab}	2.44
	10	1.11±0.01^{bc}	1.27	2.01±0.03^{abc}	1.66	2.22±0.02^{ab}	1.08	3.25±0.04^{ab}	1.17
	15	0.98±0.02^{bcd}	1.59	1.79±0.06^{abcd}	3.42	1.99±0.04^{ab}	1.99	3.12±0.01^{ab}	0.37
	20	0.86±0.02^{bcd}	2.05	1.64±0.03^{abcd}	1.85	1.65±0.02^{ab}	1.55	3.05±0.01^{ab}	0.32
	30	0.72±0.01^{bcd}	1.96	1.44±0.04^{abcd}	2.78	1.26±0.02^{ab}	1.57	2.85±0.04^{ab}	1.46
100	5	1.20±0.02^{bcd}	1.53	2.12±0.05^{abcd}	1.40	2.56±0.06^{ab}	2.43	3.28±0.02^{ab}	0.60
	10	0.99±0.02^{cd}	1.92	1.85±0.08^{abcd}	2.95	1.97±0.02^{b}	1.08	3.18±0.03^{ab}	0.91
	15	0.81±0.02^{cde}	2.19	1.68±0.04^{abcd}	4.84	1.67±0.03^{b}	1.87	3.05±0.02^{ab}	0.58
	20	0.73±0.01^{cde}	1.74	1.51±0.02^{bcd}	2.62	1.39±0.02^{bc}	1.26	2.91±0.04^{ab}	1.38
	30	0.61±0.01^{cdef}	1.05	1.35±0.02^{bcd}	1.21	1.11±0.06^{bcd}	5.10	2.68±0.03^{ab}	1.14
105	5	1.11±0.01^{cdef}	0.63	2.04±0.03^{bcd}	1.28	2.47±0.02^{bcd}	0.66	3.20±0.03^{ab}	0.82
	10	0.84±0.01^{def}	1.43	1.73±0.01^{cd}	0.82	1.77±0.01^{bcd}	0.60	3.08±0.05^{ab}	1.79
	15	0.64±0.01^{ef}	1.54	1.46±0.02^{cd}	1.45	1.37±0.01^{cd}	0.77	2.92±0.03^{b}	1.02
	20	0.53±0.01^{f}	1.74	1.30±0.02^{d}	1.25	1.16±0.02^{d}	1.58	2.76±0.03^{b}	1.28
	30	0.45±0.01^{f}	2.51	1.21±0.01^{d}	0.82	0.95±0.01^{d}	1.20	2.23±0.06^{b}	2.29

Values represent mean±standard deviation (n = 3), *initial concentrations, RSD – relative standard deviation of 3 individual measurements
Values with different letters within columns are statistically different at $p < 0.05$ using Tukey's test

Table 2
Content of individual anthocyanins (mg/kg) in sugar bilberry jam during processing

Temp. (°C)	Time (min)	Delphinidin-3-*O*-galactoside	RSD (%)	Delphinidin-3-*O*-glucoside	RSD (%)	Cyanidin-3-*O*-galactoside	RSD (%)	Delphinidin-3-arabinoside	RSD (%)	Cyanidin-3-*O*-glucoside	RSD (%)
		177±7^{a*}	4.01*	198±4^{a*}	2.14*	67±1^{a*}	2.10*	32.7±0.7*	2.21*	118±1^{a*}	1.20*
90	5	127±6^{ab}	4.46	133±7^{a}	5.31	62±2^{a}	3.44	24.7±0.9	3.49	100±1^{ab}	1.42
	10	110±4^{abc}	3.87	132±4^{a}	3.21	61±1^{a}	2.08	22.5±0.8^{a}	3.76	99±1^{ab}	1.15
	15	98±7^{abcd}	7.18	130±8^{ab}	6.55	59±1^{a}	2.27	21.9±0.6^{a}	2.91	98±4^{ab}	4.34
	20	95±1^{bcde}	1.48	122±3^{abc}	2.33	56±1^{a}	1.78	20.0±0.8^{a}	4.24	96±1^{ab}	1.48
	30	92±4^{cde}	4.63	120±1^{abcd}	1.18	49±2^{a}	4.02	18.8±0.9	4.59	95±1^{abc}	1.04
95	5	55±1^{cde}	2.57	75±3^{abcd}	3.80	27±1^{ab}	2.83	9.4±0.7^{b}	7.52	44±1^{abc}	2.59
	10	51±3^{cde}	5.50	64±4^{abcd}	6.69	25±1^{ab}	0.58	8.9±0.4^{b}	4.91	42±1^{abc}	2.35
	15	50±3^{cde}	5.61	63±3^{bcd}	4.48	24±0^{ab}	1.17	8.7±0.3^{b}	3.25	41±1^{abc}	2.73
	20	47±3^{cde}	6.36	55±1^{bcd}	2.56	23±1^{ab}	2.94	7.9±0.4^{b}	5.20	39±1^{abc}	2.90
	30	43±1de	3.26	53±2^{bcd}	4.00	21±1^{ab}	4.71	7.4±0.2^{b}	3.25	38±1^{abc}	3.73
100	5	50±1de	2.85	70±1^{bcd}	1.82	24±1^{ab}	5.93	9.2±0.3^{b}	3.22	42±2^{abc}	5.07
	10	42±1^{e}	3.37	62±1^{cd}	2.17	19±0^{b}	0.75	7.2±0.1^{b}	1.96	40±1^{abc}	3.17
	15	39±3^{e}	7.27	55±1^{cd}	1.80	17±1^{bc}	4.26	6.9±0.4^{b}	5.12	34±1^{abc}	3.91
	20	33±2^{e}	5.93	48±1^{cd}	2.97	14±1^{bc}	3.08	5.4±0.4^{bc}	7.86	32±1^{abc}	3.08
	30	23±1^{e}	3.05	39±1^{cd}	3.59	12±1^{bc}	1.15	4.4±0.1^{bc}	3.21	28±1^{abc}	7.04
105	5	47±1^{e}	3.00	58±1^{cd}	2.46	23±1^{bc}	3.12	8.8±0.3^{bc}	3.21	31±1^{abc}	1.39
	10	40±3^{e}	7.02	43±3^{cd}	6.56	19±1^{bc}	5.28	8.3±0.3^{bc}	3.42	30±1^{bc}	4.73
	15	31±1^{e}	4.09	42±1^{cd}	3.33	16±1^{bc}	3.01	7.1±0.1^{bc}	1.99	26±1^{c}	3.35
	20	26±2^{e}	6.62	38±3^{cd}	7.45	14±1^{bc}	4.91	5.7±0.1^{bc}	2.50	25±1^{c}	4.99
	30	23±1^{e}	6.23	35±1^{d}	4.05	12±0^{c}	1.14	3.0±0.1^{c}	4.70	22±1^{c}	4.47

Table 2 (Continuated)

Temp. (°C)	Time (min)	Petunidin-3-galactoside	RSD (%)	Cyanidin-3-*O*-arabinoside	RSD (%)	Peonidin-3-galactoside	RSD (%)	Petunidin-3-*O*-arabinoside	RSD (%)	Malvidin-3-galactoside	RSD (%)
		121±4^{a*}	3.50*	25.1±0.7^{a**}	2.87**	46.6±0.6^{a**}	1.24**	107±2^{a**}	1.72**	24.8±0.6^{a**}	2.34**
90	5	104±7^{ab}	6.83	19.2±0.9^{a}	4.49	44.8±0. 4^{ab}	0.98	100±2^{a}	1.69	21.0±0.4^{a}	2.09
	10	100±4^{ab}	4.26	18.8±0.8^{a}	4.51	44.1±0.9^{ab}	1.96	93±1^{ab}	1.06	18.7±0.5^{a}	2.72
	15	99±6^{b}	5.72	17.7±0.6^{a}	3.59	43.2±0.6^{b}	1.47	82±2^{ab}	2.25	16.0±0.6^{a}	3.97
	20	96±3^{bc}	2.94	16.0±0.4^{ab}	2.39	38.5±0.7^{b}	1.73	77±1^{b}	1.84	14.1±0.4^{a}	2.71
	30	82±1^{bcd}	1.72	15.6±0.9^{b}	5.53	31.9±0.9^{b}	2.71	65±1^{bc}	1.08	10.3±0.6^{a}	5.61
95	5	42±3^{cd}	6.76	6.4±0.2^{c}	3.32	12.8±0.7^{bc}	5.52	33±1^{bc}	1.70	6.6±0.2^{b}	3.23
	10	41±2^{cde}	5.16	5.3±0.2^{c}	2.95	12.7±0.4^{bc}	3.45	32±1^{bc}	3.06	6.2±0.2^{b}	2.52
	15	40±3de	6.83	4.4±0.3^{cd}	6.43	12.2±0.3^{c}	2.44	31±1^{bc}	2.67	5.5±0.3^{bc}	5.14
	20	38±1de	3.77	4.0±0.2^{cd}	3.89	11.8±0.4^{c}	3.48	29±1^{bcd}	3.96	5.1±0.2^{bc}	3.04
	30	34±2de	6.20	3.9±0.2^{cd}	6.16	11.5±0.2^{c}	2.09	27±1^{bcde}	5.18	4.6±0.2^{bcd}	5.23
100	5	40±1de	3.14	3.8±0.2^{cd}	4.10	12.0±0.3^{c}	2.47	33±1^{bcde}	2.15	6.1±0.2^{bcd}	2.57
	10	33±1de	4.07	4.0±0.1^{cd}	3.54	10.8±0.1^{c}	1.31	29±1^{bcde}	4.37	4.7±0.1^{bcd}	3.02
	15	27±1de	3.64	3.4±0.2^{cd}	6.31	9.3±0.4^{c}	3.82	25±1^{bcde}	1.41	4.0±0.2^{bcd}	5.29
	20	24±1de	5.89	2.7±0.2^{d}	6.81	7.4±0.4^{c}	5.73	21±1^{bcde}	4.63	3.4±0.2^{bcde}	5.49
	30	19±1^{def}	7.39	2.1±0.1^{d}	6.87	6.9±0.1^{c}	2.05	18±1^{bcde}	3.15	2.8±0.1^{bcde}	5.03
105	5	41±1^{def}	3.47	3.9±0.2^{d}	5.38	10.6±0.7^{cd}	6.68	28±1^{cde}	2.57	5.9±0.2^{bcde}	3.60
	10	26±1^{def}	2.76	3.7±0.2^{d}	4.61	9.8±0.2^{cd}	2.45	25±1de	2.86	4.6±0.2^{cde}	3.71
	15	22±1^{def}	6.31	3.4±0.2^{d}	5.82	8.4±0. 5^{cd}	5.76	22±1^{e}	3.35	4.2±0.2^{de}	4.67
	20	19±0^{ef}	1.50	3.2±0.1^{d}	4.01	8.0±0.1^{cd}	1.77	16±1	4.25	3.7±0.1^{de}	3.48
	30	17±1^{f}	4.17	2.8±0.1^{d}	3.61	7.0±0.1^{d}	2.03	11±1	4.65	2.7±0.1^{e}	3.72

Values represent mean±standard deviation (n = 3), *initial concentrations, RSD – relative standard deviation of 3 individual measurements
Values with different letters within columns are statistically different at $p < 0.05$ using Tukey's test

Table 3
Content of individual anthocyanins (mg/kg) in sugar-low bilberry jam during processing

Temp. (°C)	Time (min)	Delphinidin-3-*O*-galactoside	RSD (%)	Delphinidin-*O*-3-glucoside	RSD (%)	Cyanidin-3-*O*-galactoside	RSD (%)	Delphinidin-3-arabinoside	RSD (%)	Cyanidin-3-*O*-glucoside	RSD (%)
		173±7^{a*}	4.09*	195±4^{a} *	2.18*	100±1^{a*}	1.41*	40.0±0.6^{a*}	1.45*	158±1^{a*}	0.90*
90	5	159±4^{a}	2.67	185±7^{ab}	3.82	99±2^{a}	2.13	37.5±0.9^{ab}	2.30	156±1^{a}	0.91
	10	154±3^{a}	1.93	179±4^{ab}	2.36	98±1^{a}	1.29	35.3±0.5^{b}	1.44	154±1^{a}	0.73
	15	145±7^{ab}	4.88	174±5^{ab}	2.60	96±1^{a}	1.40	32.4±0.6^{bc}	1.96	148±4^{a}	2.86
	20	141±1^{abc}	1.00	171±3^{abc}	1.65	92±1^{a}	1.08	30.9±0.7^{bc}	2.15	144±1^{a}	0.98
	30	136±4^{abc}	2.60	163±2^{abc}	1.39	88±2^{a}	2.24	27.6±0.9^{bcd}	3.13	135±1^{a}	0.74
95	5	146±1^{abc}	0.97	178±3^{abc}	1.59	97±1^{ab}	0.73	36.9±0.5^{bcd}	1.34	155±1^{a}	0.73
	10	142±4^{abc}	2.99	169±4^{abc}	2.52	90±1^{ab}	1.56	34.1±0.4^{bcd}	1.29	142±1^{a}	0.70
	15	130±3^{abc}	2.18	152±2^{bc}	1.40	81±1^{ab}	0.91	33.5±0.5^{cd}	1.35	126±1^{a}	0.90
	20	128±4^{abc}	3.34	146±3^{c}	2.04	79±1^{ab}	1.60	31.0±0.7^{cd}	2.24	123±1^{a}	0.92
	30	127±2^{abc}	1.23	139±2^{c}	1.53	75±1^{ab}	1.32	29.6±0.2^{cd}	0.81	117±1^{a}	1.20
100	5	131±1^{abc}	1.08	121±1^{c}	1.05	70±1^{ab}	2.02	25.3±0.3^{de}	1.17	100±2^{a}	2.13
	10	120±1^{bc}	1.18	116±1^{c}	1.16	63±1^{b}	1.59	23.8±0.6^{ef}	2.44	95±1^{ab}	1.34
	15	92±3^{bc}	3.07	106±1^{c}	0.93	58±1^{b}	1.96	22.8±0.4^{ef}	1.55	90±1^{ab}	1.49
	20	90±2^{bc}	2.20	100±1^{cd}	1.41	54±1^{b}	1.39	20.7±0.5^{ef}	2.25	89±1^{abc}	1.11
	30	86±2^{bc}	2.48	91±1^{cd}	1.55	51±1^{b}	1.42	18.2±0.1^{efg}	0.78	88±2^{abc}	2.24
105	5	100±1^{bc}	1.41	117±2^{cd}	1.81	68±1^{b}	1.05	23.2±0.7^{efg}	3.05	92±1^{abc}	0.77
	10	94±2^{c}	1.81	108±3^{cd}	2.62	53±1^{b}	1.59	21.5±0.3^{fg}	1.45	87±1^{abc}	1.62
	15	88±1^{c}	1.44	98±1^{cd}	1.45	50±1^{b}	1.15	19.2±0.6^{g}	3.10	82±0^{bc}	0.35
	20	85±2^{c}	2.00	96±3^{cd}	2.96	49±1^{bc}	1.45	18.1±0.2^{g}	1.09	80±1^{bc}	1.58
	30	75±1^{c}	1.88	91±1^{d}	1.56	48±1^{c}	1.20	17.4±0.1^{g}	0.81	78±1^{c}	1.26

Table 3 (Continuated)

Temp. (°C)	Time (min)	Petunidin-3-galactoside	RSD (%)	Cyanidin-3-*O*-arabinoside	RSD (%)	Peonidin-3-galactoside	RSD (%)	Petunidin-3-*O*-arabinoside	RSD (%)	Malvidin-3-galactoside	RSD (%)
		171±5[a]*	3.05	31.4±0.4*	1.40	79.7±0.9[a]*	1.08	170±5*	2.75	44.1±0.7*	1.64
90	5	168±6[ab]	3.79	27.1±0.3[a]	1.09	77.2±0.4[a]	0.57	161±5[a]	2.82	37.9±0.4[a]	1.16
	10	166±4[ab]	2.55	25.8±0.7[a]	2.52	74.8±0.9[a]	1.15	155±4[ab]	2.55	37.0±0.3[a]	0.92
	15	161±6[ab]	3.52	23.9±0.8[a]	3.26	72.3±0.8[a]	1.08	149±3[ab]	2.19	35.1±0.6[a]	1.81
	20	157±3[ab]	1.80	23.5±0.4[a]	1.62	72.0±0.8[a]	1.12	140±1[abc]	1.01	33.4±0.8[ab]	2.41
	30	136±4[ab]	2.80	22.9±0.9[a]	3.77	67.9±0.9[a]	1.27	123±4[abc]	2.85	31.1±0.4[ab]	1.41
95	5	162±3[ab]	1.75	27.9±0.4[a]	1.27	74.2±0.9[a]	1.24	155±5[abc]	3.46	37.3±0.8[ab]	2.09
	10	148±2[abc]	1.43	24.6±0.7[a]	2.93	69.5±0.6[a]	0.83	149±2[abc]	1.62	36.4±0.3[abc]	0.82
	15	142±2[abc]	1.19	22.2±0.2[a]	0.77	63.2±0.7[a]	1.14	140±3[bc]	1.82	34.7±0.3[abc]	0.82
	20	138±4[bc]	3.17	21.0±0.6[a]	2.76	61.4±0.4[a]	0.67	138±4[bc]	2.88	34.1±0.4[abc]	1.29
	30	134±2[bc]	1.59	20.1±0.2[a]	1.20	58.6±0.7[ab]	1.13	121±1[c]	1.17	30.3±0.2[bc]	0.79
100	5	104±3[c]	2.59	23.0±0.6[a]	2.52	58.3±0.6[ab]	1.09	103±2[c]	2.07	28.8±0.2[bc]	0.54
	10	96±1[c]	1.40	21.8±0.1[a]	0.65	47.5±0.9[ab]	1.82	93±1[cd]	1.37	22.0±0.3[cd]	1.35
	15	89±1[cd]	1.11	19.3±0.2[a]	1.10	45.8±0.4[b]	0.77	86±3[cd]	3.21	21.0±0.3[de]	1.39
	20	87±1[cd]	1.62	18.1±0.9[a]	4.93	42.2±0.4[b]	1.01	83±5[cde]	6.30	20.4±0.2[de]	0.88
	30	86±3[cd]	3.44	17.3±0.1[a]	0.82	40.2±0.1[bc]	0.35	80±2[cde]	2.46	18.8±0.1[de]	0.75
105	5	94±3[cd]	3.15	20.0±0.2[a]	1.06	53.4±0.7[bc]	1.32	100±4[cde]	3.53	21.5±0.2[de]	0.99
	10	91±2[cd]	2.33	16.8±0.2[a]	1.01	43.9±0.7[bc]	1.51	93±1[cde]	1.53	23.9±0.2[de]	0.71
	15	84±1[d]	1.68	15.6±0.3[a]	2.18	40.0±0.5[c]	1.20	83±2[de]	2.04	20.8±0.6[de]	2.99
	20	81±2[d]	2.09	14.9±0.6[a]	3.90	38.9±0.1[c]	0.36	78±5[de]	5.82	18.6±0.6[e]	3.12
	30	80±2[d]	2.66	14.3±0.3[a]	2.08	36.7±0.1[c]	0.39	76±3[e]	3.72	17.2±0.7[e]	4.19

Values represent mean±standard deviation (n = 3), *initial concentrations, RSD – relative standard deviation of 3 individual measurements
Values with different letters within columns are statistically different at $p < 0.05$ using Tukey's test

Heating at 105°C for 30 min brought to severe loss in contents of all identified anthocyanins. Retention percentages of cyanidin-3-*O*-galactoside, peonidin-3-galactoside, petunidin-3-galactoside, delphinidin-3-*O*-galactoside and malvidin-3-galactoside were 17.9, 15, 14.0, 13 and 10.9% in sugar jam and 48.0, 46.0, 44.7, 43.3 and 39.0% in sugar-low jam, respectively.

Cyanidin-3-*O*-glucoside retained better with 18.6% than delphinidin-3-*O*-glucoside with 17.7% in sugar jam. The same was observed in a sugar-low jam where 49.4% of cyanidin-3-*O*-glucoside and 46.7% of delphinidin-3-*O*-glucoside were retained.

The highest retention among arabinosides was observed at cyanidin-3-*O*-arabinoside, followed by petunidin-3-*O*-arabinoside and delphinidin-3-arabinoside and yielding 11.1, 10.3 and 9.2% in sugar jam and 45.5, 44.7 and 43.5% in a sugar-low jam.

Cyanidin-3-*O*-galactoside and cyanidin-3-*O*-glucoside were found to be the most stable, while petunidin-3-*O*-arabinoside and delphinidin-3-arabinoside were the least stable identified anthocyanins.

Our results are in agreement with a study of Trošt et al. [28] on anthocyanin degradation during storage of blueberry-aronia nectar, where the stability of individual anthocyanins with respect to aglycone was as follows: cyanidin > peonidin > petunidin > malvidin > delphinidin.

The obtained results have also shown that galactosides and glucosides retained better than arabinosides, which is in accordance with the previously mentioned study [28] where larger hexose sugars exhibit greater stability than smaller pentose sugars.

Degradation kinetics of anthocyanins

Linear regression showed that the degradation of total anthocyanins in both sugar and sugar-low jam samples followed by a first-order reaction (Figure 1 and Figure 2). In this case, a first-order reaction can generally be expressed using Eq. 1 [29, 30] where c is the anthocyanin concentration at time t, c_o is the initial concentration of total anthocyanins (mg/g), t is the treatment time (min) and k the first-order degradation rate constant (min^{-1}).

$$c = c_0 \cdot \exp(-k \cdot t) \quad (1)$$

The half-lives ($t_{1/2}$) of the anthocyanins were calculated by the Eq. 2:

$$t_{1/2} = -\ln(0.5)/k = 0.693/k \quad (2)$$

The temperature-dependence degradation rate constant was represented by the Arrhenius, Eq. 3:

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

where k_o is the frequency factor, E_a is the activation energy, R is the universal gas constant, and T is the absolute temperature.

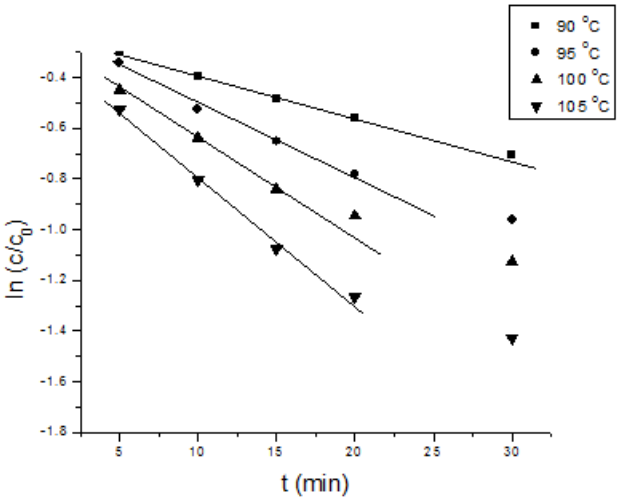

Figure 1. Degradation of total anthocyanins in sugar bilberry jam samples

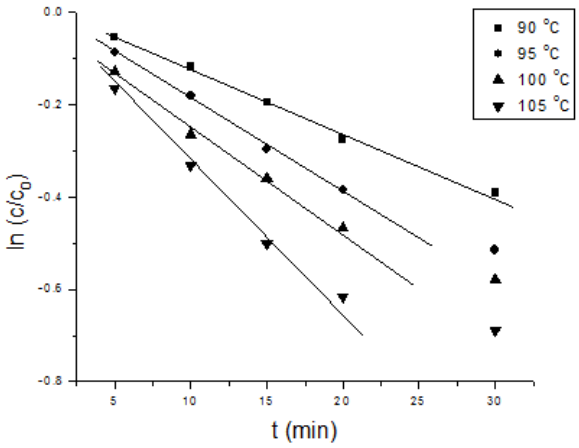

Figure 2. Degradation of total anthocyanins in sugar-low bilberry jam samples

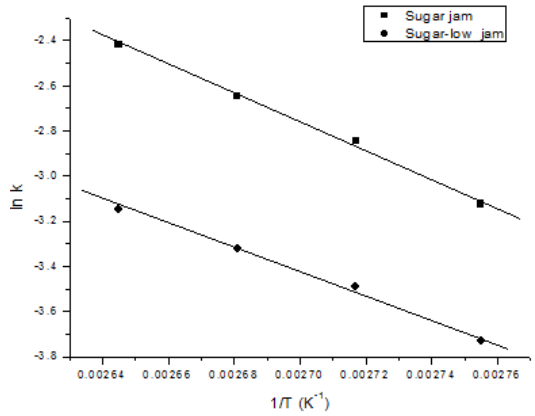

Figure 3. The Arrhenius plot for degradation of total anthocyanins in bilberry jam samples

Effect of the heating temperature on the total anthocyanin degradation rate is shown in Figure 1 and Figure 2, and the kinetic parameters are given in Table 4. The rate constant obeyed the Arrhenius relationship (Eq. 3), and the Arrhenius plot is given in Figure 3. The high R^2 values obtained from the Arrhenius plot confirm that the degradation increased with increased temperature and time ($0.9724<R^2<0.9978$). Therefore, the percentage of retention of total anthocyanins significantly depends on heating temperature and was ranged from 49.5% to 73.9 at 90 °C, from 38.3 to 70.7% at 95°C, from 32.4 to 63.8% at 100°C and from 23.9 to 59.0% at 105°C in sugar jam formulation. Total anthocyanins have shown greater resistance towards high temperatures exposure in the sugar-low formulation, yielding from 67.6 to 94.6% at 90°C, from 59.7 to 91.7% at 95°C, from 56.0 to 88.0% at 100°C and from 50.2 to 84.6% at 105°C.

Table 4
Effect of heating temperature on the reaction constant rate (k), time of half-life ($t_{1/2}$) and activation energy (E_a) values of total anthocyanins degradation in sugar and sugar-low bilberry jams

Sugar jam				
Temperature (°C)	$k\times10^2$ (min^{-1})	R^2	$t_{1/2}$ (min)	E_a (kJ/mol)
90	4.4±0.3	0.9948	15.7	51±1
95	6.0±0.3	0.9857	11.5	
100	7.1±0.5	0.9724	9.8	
105	8.9±0.4	0.9908	7.8	
Sugar-low jam				
Temperature (°C)	$k\times10^2$ (min^{-1})	R^2	$t_{1/2}$ (min)	E_a (kJ/mol)
90	2.4±0.1	0.9947	28.4	40±1
95	3.2±0.2	0.9978	21.8	
100	3.6±0.1	0.9889	19.1	
105	4.3±0.2	0.9904	16.2	

The larger amount of sugar in sugar jam gave a two-fold increase in degradation rate constants and an approximately two-fold decrease in the half-lives comparing to the sugar-low formulation at all applied temperatures. The $t_{1/2}$ values ranged from 15.7 to 7.8 min for sugar jam and from 28.4 to 16.2 min for the sugar-low jam at 90, 95, 100 and 105°C, respectively.

The value of the activation energy was 40 kJ/mol and 51 kJ/mol for low-sugar and sugar jam, respectively. This finding shows that the degradation of anthocyanins is more affected by temperature elevation in sugar formulation. Obtained values are in agreement with the results of Dyrby et al. [31] on thermal degradation of anthocyanins in soft drink mediums of elderberry juice and blackcurrant pomace extracts (56 kJ/mol and 50 kJ/mol), but lower than the ones reported in a study of Wang and Xu [13] on degradation kinetics of anthocyanins in blackberry juice and concentrate (65.06 kJ/mol and 75.5 kJ/mol) and in a study of Kechinski et al. [14] on degradation kinetics of anthocyanins in blueberry juice (80.4 kJ/mol).

Obtained results are in agreement with the results reported by de Moura et al. [15], where it was found that reduction in the amounts of anthocyanin compounds was greater in the sugar jam. In a study conducted by de Rosso and Mercandante [32], it was also shown that addition of sugars exhibited an adverse effect on the stability of anthocyanins from tropical fruits. In general, these results are in accordance with the pioneering work of Meschter [33], where it was illustrated the ability of some sugars and sugar degradation products to increase the rate of strawberry pigment loss. Also, thermal degradation of anthocyanins includes the chalcone formation or loss of glycosyl moieties [34] (Nayak et al., 2011). In relation to the stability, anthocyanins may suffer reactions that altered their structures due to the electronic deficiency of their flavylium nuclei [35] (Turturica et al., 2018).

4.Conclusions

This study showed that significant changes occur in total and individual anthocyanin contents when bilberry fruits are processed into sugar and sugar-low jams. The main practical application of this work is that it showed that larger amounts of total and individual anthocyanins are retained when jams are prepared at lower temperatures. It also showed that the presence of sugar had a negative effect on total and individual anthocyanins, as well as on total polyphenols, flavonoids and antioxidant activity of bilberry jams. According to our knowledge at this moment, this is the first work that comparatively studied degradation kinetics of anthocyanins at high temperatures in bilberry jams, immediately after preparation. Thermal degradation of bilberry anthocyanins followed first-order reaction kinetics. Higher retention of all analyzed compounds was accomplished in the sugar-low jam, in lower temperatures and shorter heating time.

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