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Research Article

Journal of Food Quality Thermal Degradation of Plum Anthocyanins: Comparison of Kinetics from Simple to Natural Systems

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The stability of anthocyanin was assessed over a temperature range of 50–120°C in different simulated plum juices in order to compare the thermal behavior in the presence of certain compounds. The results were correlated with the antioxidant activity and intrinsic fluorescence spectra. The results suggested significant changes, especially at higher temperature; hence, increase in the fluorescence intensity and some bathochromic and hypsochromic shifts were observed. Anthocyanins in natural matrices presented the highest rate for degradation, followed by the anthocyanins in juices with sugars. Values of the activation energies were 42.40 ± 6.87 kJ/mol for the degradation in water, 40.70 ± 4.25 kJ/mol for the juices with citric acid, 23.03 ± 3.53 kJ/mol for the juices containing sugars, 35.99 ± 3.60 kJ/mol for simulated juices with mixture, and 14.19 ± 2.39 kJ/mol for natural juices. A protective effect of sugars was evidenced, whereas in natural matrices, the degradation rate constant showed lower temperature dependence.

1. Introduction

Fruit-based foods have turned into very popular goods because consumers associate them to healthy products, so their commercialization has increased in the last years [1]. Among them, fruits like red plums are one of the most important because they are consumed directly or are used as a raw material for juices, puree, jellies, compote, and jams. Besides other sensorial parameters, the color is one of main quality parameter, which influences the consumers' preference and behavior. The attractive red color of plums is associated with the presence of anthocyanins. Many studies demonstrated that anthocyanins have potential health benefits for humans as they possess anticarcinogenic properties [2], prevent cardiovascular diseases like atherosclerosis [3], have antidiabetic properties [4], and protect against Alzheimer's disease [5]. Additionally, the special interest in anthocyanins is early recognized if as natural food colorants, especially if suitable purified and stable material becomes commercially available [6].

Anthocyanins are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium or flavilium salts. Glycosylation and acylation of the aglycone moieties (mainly six anthocyanidins: pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin) by different sugars and acids, at different positions, account for the broad structural diversity of these pigments [7]. The main anthocyanins present in red plums are 3-O bound glucose of cyanidin and peonidin and 3-O bound rutin of cyanidin and peonidin [8]. However, the total content of the anthocyanins in red plums are affected by the cultivar, maturity level, year of production, and different environmental factors [9].

Therefore, one of the main challenges in red plum product processing is the preservation of anthocyanins which is an important issue because anthocyanins are highly unstable and

are susceptible to degradation [10]. The stability of the anthocyanins depends on several factors such as pH, temperature, oxygen, metallic ions, ascorbic acid, sugars, and their degradation products [11]. It has been reported that anthocyanins exhibit higher stability under acidic conditions, but under normal processing and storage conditions readily convert to colorless derivatives and subsequently to insoluble brown pigments [7]. The red plum juice color is influenced by thermal treatment used to preserve and to extend the shelf life of juices. During thermal treatment, the anthocyanins degrade due to their high reactivity and also they may polymerize which is related to degradation of color and loss of nutritional values in food products [12]. Several studies were performed in order to evaluate the stability of anthocyanins through addition of sugars, acids, hydrocolloids, salts, and different phenolic compounds in various fruit products during thermal treatment [12–15]. By studying the data from the literature, it can be assumed that the thermal degradation of anthocyanins depends on matrices from which it originates and the complexity of the heating environment. It is clear that the raw materials' variability and the different heating conditions are hardly comparable in terms of temperature and time, solid content, or the presence of extraction solvent [16]. There is a need to relate the kinetics of thermal degradation of anthocyanins concomitant to increase the complexity of the system. Therefore, the main objective was the evaluation of thermal degradation of anthocyanins from plums in medium with increased complexity during heating at a temperature range between 50 and 120°C on a kinetic basis. The HPLC technique was employed to evaluate the degradation of individual anthocyanins identified in plum juices at temperatures of 25°C and 100°C, after 20 minutes of holding. Since red plum juices are a good source of antioxidants, the change in antioxidant capacity during thermal treatment was also investigated. Intrinsic fluorescence spectroscopy technique was used as an additional technique to extend the possibilities of analysis of the heat-induced changes in anthocyanins.

2. Materials and Methods

2.1. Chemicals. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), sodium acetate, potassium chloride, ethanol, and methanol (HPLC grade) were obtained from Sigma Aldrich Steinheim, Germany. Cyanidin and pelargonidin standards were obtained from Extrasynthèse (Z.I Lyon Nord, France).

2.2. Juices Preparation. Plums (*Prunus domestica, Vanette* variety) were provided from the local market (Galaţi county) during June–July, 2015. Fruits samples were washed with water in the ratio of 1 : 2 (w/w). After washing, the skins were manually separated and then were washed with distilled water. Finally, they were blotted on paper towels to remove any residual pulp. The extraction of anthocyanins from freeze-dried red plums was performed according to a previously described procedure [8].

Plum juices were obtained as following:

(i) PW (plum juice in water) was obtained from the concentrated plum extract (lyophilized plum skins: 70% ethanol ratio of 1 : 8) which was then dissolved in 20 mL distilled water.

(ii) PCA (plum juice with citric acid) was obtained in the same way as PW, the difference being the addition of citric acid (22.13 g citric acid/L extract).

(iii) PS (plum juice with sugars) was obtained in the same way as PW with the addition of glucose and fructose (244 g glucose/L extract and 61.97 g fructose/L extract).

(iv) PM (plum juice with mixture) represents the juice obtained with the addition of all the above mentioned compounds in the same ratio.

(v) PN (natural plum juice) was obtained from 250 g plums that were crushed and afterwards 1 g of Zymorouge enzyme was added and left for 24 h in order to achieve the extraction of the pigments. The obtained juice was then filtered using a cheese cloth.

2.3. Heat Treatment. A volume of 200 μL of juices was filled in Eppendorf tubes (1 cm diameter) and heated at temperatures ranging from 50 to 120°C for 30 min for the fluorescence spectroscopy experiments. The same temperature range from 50°C to 120° but different times (0–60 min) were used for thermal treatment in the case of thermal degradation kinetic studies.

Heating experiments were conducted in a thermostatic oil bath (Nahita 602/3, Navarra, Spain) according to a previously described procedure [8].

2.4. HPLC Analysis of Anthocyanins. The identification and quantification of plum juice anthocyanins were made using a Thermo Finnigan Surveyor HPLC system, controlled by Xcalibur software system (Finnigan Surveyor LC, Thermo Scientific, USA). The column used for the separation of plum anthocyanins was Synergi 4u Fusion-RP 80A (150 mm × 4.6 mm, 4 μm). The column was operated at 25°C, at a wavelength of 520 nm. The elution profile was accomplished with 100% methanol (A) and 10% formic acid (B). The elution program employed was 0–20 min, 9–35% (A); 20–30 min, 35% (A); 30–40 min, 35–50% (A); and 40–55 min, 50–9% (A). The injection amount was 10 μL at a flow rate of 1 mL/min.

2.5. Phytochemicals Analysis. Total monomeric anthocyanins (TAC) and free radical scavenging activity (DPPH RSA) of juices were estimated according to our earlier report [8].

2.6. Fluorescence Spectroscopy Measurements. Fluorescence measurements were carried out using a LS-55 luminescence spectrometer (PerkinElmer Life Sciences, Shelton, CT, USA) as described earlier by Turturica [8].

2.7. Mathematical Models and Kinetic Analysis. The degradation kinetics of TAC in PW, PCA, PS, and PM were described by fitting the first order kinetic model (1) to experimental data:

$$\frac{C}{C_0} = e^{-kt}, \quad (1)$$

where C is the parameter to be estimated, the subscript 0 indicates the initial value of the parameter, t is the heating time, and k is the rate constant at temperature T (1/min).

The degradation kinetic of TAC in PN was described by fitting a first-order fractional conversion kinetic model. In this model, the changes in TAC (C) as a function of heating time are described by (2):

$$C_t = C_\infty + (C_i - C_\infty)\exp(-kt), \quad (2)$$

with C_∞ the equilibrium value at infinite heating time (the value after which longer heating time does not result in changes in C value) and C_i is the TAC values of the samples at time 0 of thermal treatment.

The half-life ($t_{1/2}$) of the reaction was calculated assuming the first-order kinetics according to (3):

$$t_{1/2} = \frac{-\ln 0.5}{k}. \quad (3)$$

The Arrhenius model was used to describe the temperature dependence of degradation rate constants.

2.8. Statistical Analysis of Data. All experiments were performed in triplicates with duplicate samples. The results were expressed in terms of average values. Statistical analysis of data was performed using the data analysis tool pack of the Microsoft Excel software. The coefficient of determination (R^2) and mean square error (MSE) were used as criteria for adequacy of fit.

3. Results and Discussion

3.1. HPLC Analysis of Anthocyanins from Juices. Chromatographic analysis of the plum juices performed at 520 nm pointed out the presence of four peaks corresponding to cyanidin 3-xyloside/cyanidin 3-glucoside (peak 1), cyanidin 3-rutinoside (peak 2), peonidin 3-glucoside (peak 3) and peonidin 3-rutinoside (peak 4) (Figure 1).

The content of each anthocyanin at different temperatures corresponding to each juice is presented in Table 1.

As it can be seen from Table 1, in all plum juices, the predominant anthocyanin was C3R. Thermal treatment at 100°C for 20 minutes caused a decrease in anthocyanins content. In PW, the anthocyanin concentration decreases with about 22–25%, while in the PCA, C3G decreases with about 17% compared to the other three anthocyanins, which degrades with approximately 45%. Interesting is the increase in C3G content by approximately 8% in PS by heating at 100°C, while the other two compounds concentration decreases with 19–21%. A significant increase in C3G and P3G concentration of approximately 44% and 162%, respectively, occurred in PM, while C3R and P3R concentration decreased with 5.72% and 4.33%, respectively. It can be appreciated that the mixture between citric acid, glucose, and fructose had the most protective effect on anthocyanins thermal degradation.

Only three anthocyanins could be quantified in PN after heating at 100°C, namely, cyanidin 3-xyloside/cyanidin 3-glucoside (peak 1), cyanidin 3-rutinoside (peak 2), and peonidin 3-rutinoside (peak 4), whereas peonidin 3-glucoside (peak 3) was entirely degraded. Significant degradation of cyanidin 3-xyloside/cyanidin 3-glucoside (91.67%, peak 1), cyanidin 3-rutinoside (96.52%, peak 2), and peonidin 3-rutinoside (91.22%, peak 4) was found in PN.

3.2. The Influence of Thermal Treatment on TAC and DPPH RSA of Plum Juices. Total anthocyanin content of untreated PW, PCA, PS, PM, and PN was 144, 52, 53, 51, and 38 mg/L, respectively. Heating the PCA at temperature ranging from 50 to 90°C for up to 45 min caused an increase in TAC. This phenomenon was explained by Hubbermann [14] by the presence of an equilibrium between the four anthocyanin chromophores in aqueous solutions. Heating of juices presented a significant impact on anthocyanin content, with a decrease of 85%, 53%, 61%, 50%, and 86%, respectively, after 60 min of treatment at temperature of 120°C. It can be noticed that the presence of citric acid, sugar, and their combination had a stabilizing effect on anthocyanins thermal degradation, in accordance with previously reported results [10, 14]. The stability of anthocyanins is influenced by temperature, pH, chemical structure of the anthocyanin compound, UV light, oxygen, oxidative and hydrolytic enzymes, proteins, and the metallic ions [17]. In food industry, the citric acid is used as an acidifier and antioxidant to control the browning process [18, 19]. According to Shaheer [20], the higher thermostability of anthocyanins in the presence of citric acid may be due to diacylation of structure that improves anthocyanin stability by protecting it from hydration. They also suggested that the presence of inter- and intramolecular copigmentation with other moieties and polyglycosylated and polyacylated anthocyanins provides greater stability towards change in temperature, pH, and light.

An increase in anthocyanidin stability has been also reported by Francis [21] due to glycosylation and acylation. To the best of our knowledge, there are limited studies in the literature presenting the presence of acylation for the anthocyanins from plum. For example, Wu and Prior [22] reported the presence of cyanidin 3-(6″-acetoyl) glucoside and cyanidin 3-(maloyl) glucoside in plum and black plum. However, we suspected that PCA should contain acylated anthocyanins to a certain extent because its anthocyanins showed higher stability than those from corresponding juices. Higher stability of plum anthocyanins could, therefore, be attributed to the presence of much higher amounts of acylated anthocyanins. Stabilization effect due to sugar addition may be caused by lowering the water activity since water activity was reported in literature to influence anthocyanin stability [23].

Szalóki-Dorkó [24] suggested also a decrease of the anthocyanin content of Érdi bőtermő juices, from 812 to 501 mg/L at 90°C after 240 min of heating. Degradation was lower at 80°C, and the treatment for 4 h at this temperature resulted in 29% loss of total anthocyanin content compared to the original levels found in fresh juice. Volden et al. [25] reported that blanching, boiling, and steaming resulted in

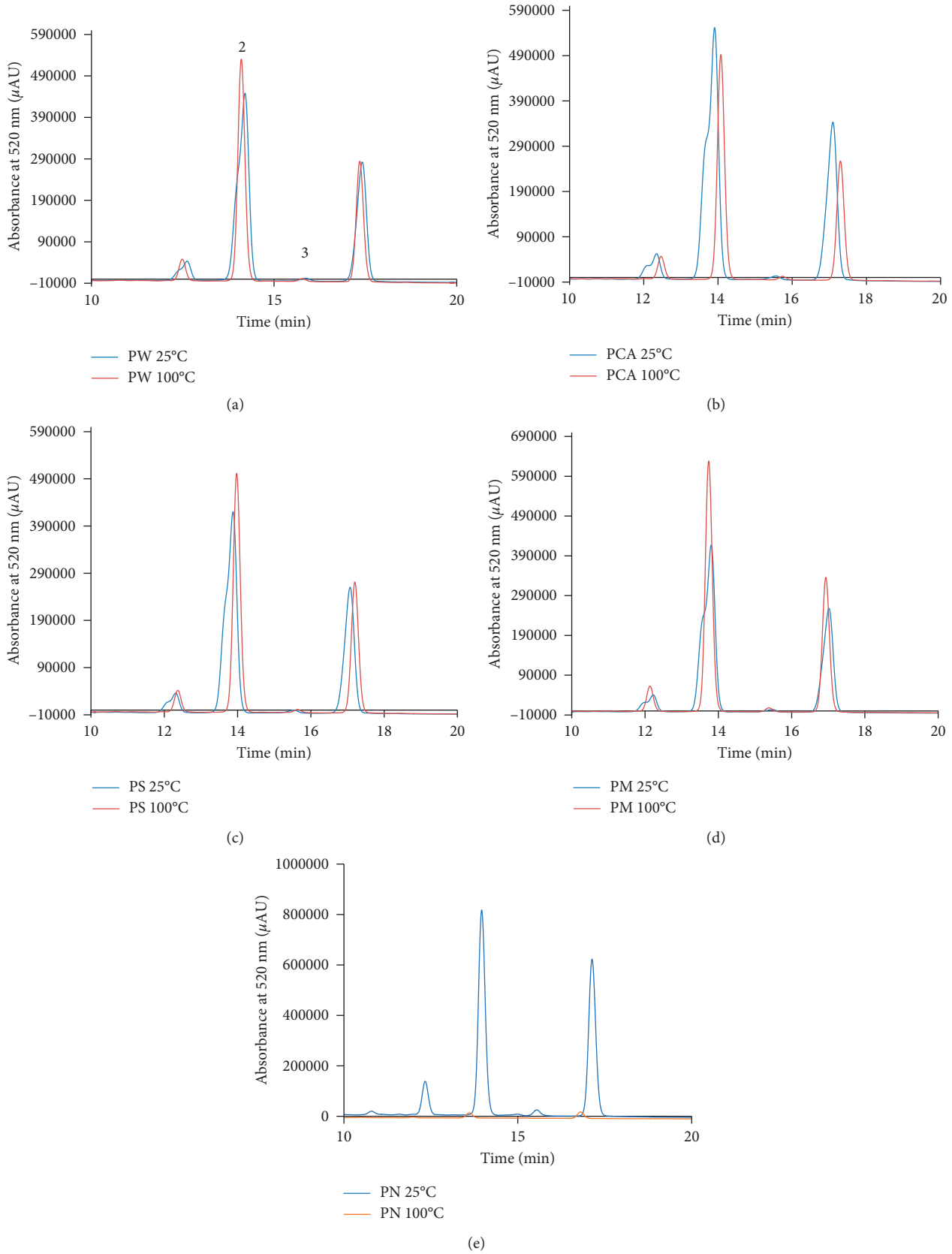

Figure 1: HPLC chromatogram of plum anthocyanins at 25°C and after 20 minutes of treatment at 100°C from (a) plum juice with water (PW), (b) plum juice with citric acid (PCA), (c) plum juice with sugars (PS), (d) plum juice with mixture (PM), and (e) natural plum juice (PN) at 520 nm. For PW, PCA, PS, and PM, peaks represent (1) cyanidin 3-glucoside; (2) cyanidin 3-rutinoside; (3) peonidin 3-glucoside; (4) peonidin 3-rutinoside. Peak assignments are given in Table 1.

Table 1: Anthocyanins content in PW, PCA. PS, PM, and PN expressed as μg/g at 25°C and after 20 min of treatment at 100°C.

Juice	PW		PCA		PS		PM		PN	
Anthocyanin	25°C	100°C	25°C	100°C	25°C	100°C	25°C	100°C	25°C	100°C
C3G	37.89 ± 2.87	28.17 ± 1.78	32.30 ± 1.25	27.02 ± 1.78	21.47 ± 1.99	23.16 ± 1.54	24.62 ± 1.41	35.40 ± 1.24	87.05 ± 1.52	7.25 ± 0.09
C3R	434.61 ± 11.54	336.42 ± 15.98	559.55 ± 52.21	309.08 ± 11.78	403.36 ± 14.32	316.47 ± 17.56	421.07 ± 16.27	396.99 ± 5.01	525.88 ± 3.14	18.30 ± 1.87
P3G	0	0	0.93 ± 0.12	0.65 ± 0.10	0	0	1.58 ± 0.45	4.16 ± 1.01	16.36 ± 0.48	0
P3R	236.78 ± 14.54	183.36 ± 10.24	300.71 ± 11.24	166.22 ± 2.36	217.21 ± 10.87	174.65 ± 9.47	224.87 ± 11.47	215.12 ± 14.21	415.54 ± 5.69	36.48 ± 2.94

C3G: cyanidin 3-xyloside/cyanidin 3-glucoside; C3R: cyanidin 3-rutinoside; P3G: peonidin 3-glucoside; P3R: peonidin 3-rutinoside.

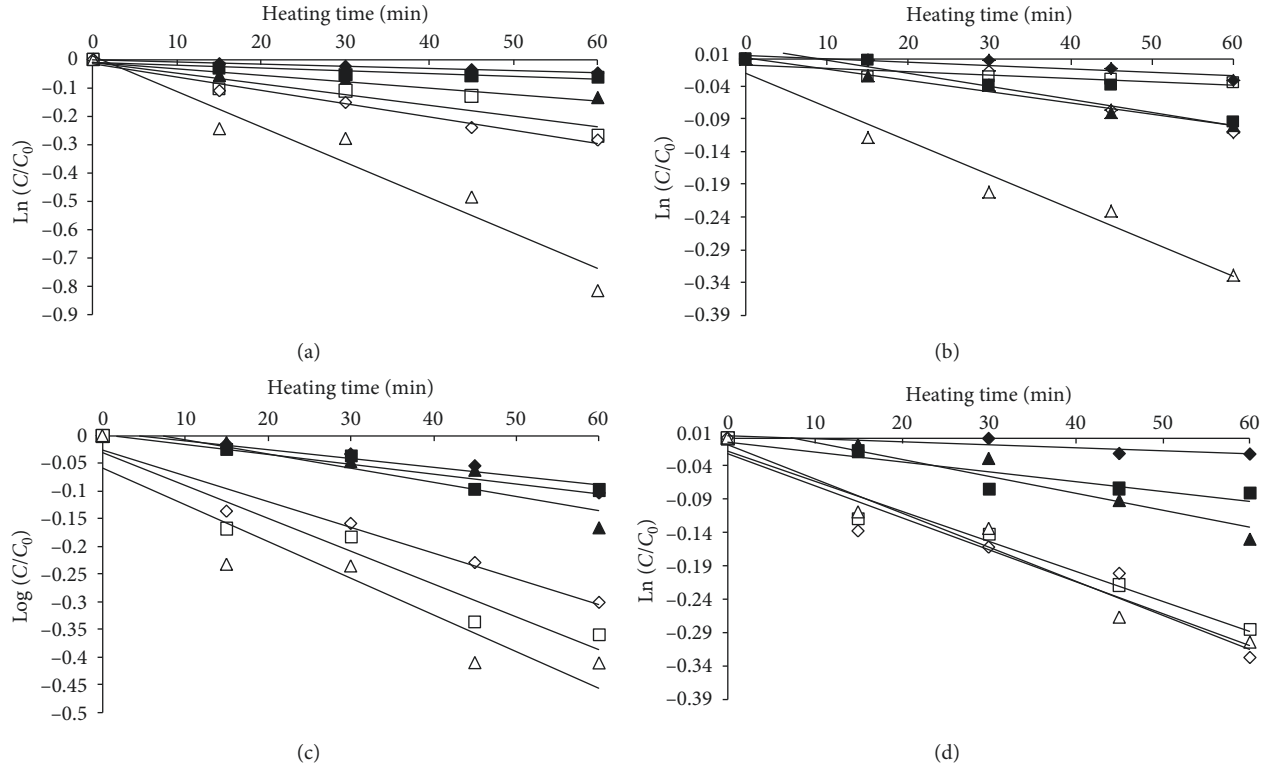

Figure 2: Isothermal degradation of anthocyanin in PW (a), PCA (b), PS (c), and PM (d) as described by first-order kinetic model at different temperatures (◆ 50°C, ■ 70°C, ▲ 90°C, □ 100°C, ◇ 110°C, and Δ 120°C).

anthocyanin losses of 59%, 41%, and 29%, respectively in red cabbage. Thermal degradation of TAC includes the chalcone formation or loss of glycosyl moieties [26].

The antioxidant activities of the five tested juices were 66.07, 25.00, 30.31, 26.34, and 52.57% in PW, PCA, PS, PM, and PN, respectively. Heating caused an increase in DPPH RSA to 83.14%, 39.70%, and 45.02%, respectively, after 15 min at 50°C. The increase in DPPH RSA may be due to the anthocyanin degradation in phloroglucinaldehyde and protocatechic acid, the latter having higher antioxidant activity [27]. At higher temperature of heating, the treatment induced a decrease in the concentrations of anthocyanins, with negative impact on antioxidant activity. However, at a higher temperature, a decrease with 8% in PW and 94.38% in PM was registered after 60 min at 120°C. A protective effect of food matrices was observed in PN, the reduction in DPPH RSA being of only 24%.

3.3. Kinetics of Thermal Degradation. Degradation kinetic of TAC in PW, PCA, PS, and PM was modeled using first-order kinetic model (2)(Figure 2), whereas the degradation of anthocyanin in PN was fitted to the fractional conversion kinetic model (3) (Figure 3).

The kinetic parameters of TAC degradation in different juices while heating are displayed in Table 2. Considering the wide sample variability and that a single set of parameters is used at each temperature for all experiments, the results showed good correlation between experimental and correlated values (Figure 4).

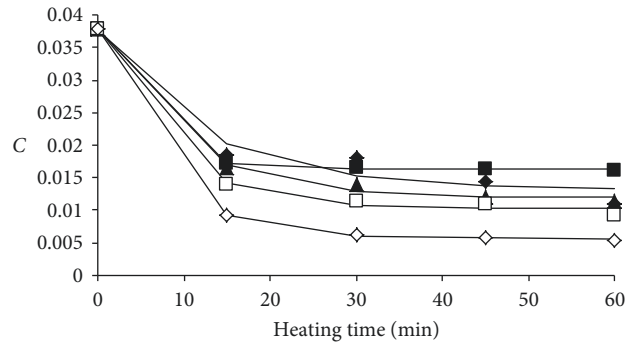

Figure 3: Isothermal degradation of TAC in PN as described by the fractional conversion kinetic model (3) at different temperatures (◆ 50°C, ■ 70°C, ▲ 90°C, □ 100°C, and ◇ 110°C). The lines represent model fits to experimental data.

The heat-induced changes in TAC were described in terms of degradation rate (1/min) and degradation energy of activation (E_a). In case of PW, the degradation rate constant (k) increases from $0.18 \pm 0.01 \cdot 10^{-2}$ 1/min at 50°C to $2.87 \pm 0.47 \cdot 10^{-2}$ 1/min at 120°C (Table 2). The degradation rate of anthocyanins in PCA ranged from $0.04 \pm 0.01 \cdot 10^{-2}$ 1/min at 50°C to $1.17 \pm 0.11 \cdot 10^{-2}$ 1/min at 120°C. For PS, the k was 3.66 times higher at 120°C when compared with 50°C, ranging from $0.36 \pm 0.01 \cdot 10^{-2}$ 1/min to $1.54 \pm 0.12 \cdot 10^{-2}$ 1/min, suggesting a lower thermal stability of anthocyanin compounds at higher temperature. An increase in k values were observed also in case of PM, varying from $0.11 \pm 0.08 \cdot 10^{-2}$ 1/min at 50°C to $1.17 \pm 0.25 \cdot 10^{-2}$ 1/min at 120°C. A higher degradation rate

Table 2: Estimated kinetic parameters (rate constant (k) and activation energy E_a) of anthocyanins thermal degradation in plum juices.

Juice	Temperature °C	$k \cdot 10^{-2}$ (min^{-1})	R^2	$t_{1/2}$ (h)	E_a (kJ/mol)	R^2
PW	50	0.18 ± 0.01[a]	0.99	6.27 ± 1.15	42.40 ± 6.87	0.90
	70	0.23 ± 0.02	0.85	5.01 ± 0.57		
	90	0.52 ± 0.05	0.94	2.18 ± 0.23		
	100	0.87 ± 0.11	0.85	1.32 ± 0.10		
	110	1.08 ± 0.07	0.98	1.06 ± 0.16		
	120	2.87 ± 0.47	0.94	0.40 ± 0.02		
PCA	50	0.04 ± 0.01	0.75	25.08 ± 1.28	40.70 ± 4.25	0.99
	70	0.11 ± 0.01	0.97	10.03 ± 0.98		
	90	0.23 ± 0.02	0.87	5.01 ± 0.57		
	100	0.34 ± 0.05	0.76	3.34 ± 1.04		
	110	0.46 ± 0.04	0.89	2.50 ± 0.69		
	120	1.17 ± 0.11	0.9	0.98 ± 0.15		
PS	50	0.36 ± 0.01[a]	0.93	3.13 ± 0.15	23.03 ± 3.53	0.91
	70	0.46 ± 0.08	0.92	2.50 ± 0.15		
	90	0.59 ± 0.11	0.85	1.92 ± 0.10		
	100	1.05 ± 0.12	0.96	1.09 ± 0.09		
	110	1.35 ± 0.17	0.93	0.85 ± 0.06		
	120	1.54 ± 0.12	0.88	0.74 ± 0.09		
PM	50	0.11 ± 0.08[a]	0.74	10.03 ± 0.38	35.99 ± 3.60	0.96
	70	0.34 ± 0.07	0.83	3.34 ± 0.16		
	90	0.57 ± 0.05	0.91	2.00 ± 0.23		
	100	1.03 ± 0.12	0.96	1.11 ± 0.09		
	110	1.10 ± 0.14	0.93	1.04 ± 0.08		
	120	1.17 ± 0.25	0.96	0.98 ± 0.04		
PN	50	8.21 ± 3.49	0.96	0.14 ± 0.003	14.19 ± 2.39	0.92
	70	10.93 ± 1.69	0.99	0.10 ± 0.006		
	90	13.04 ± 1.39	0.99	0.08 ± 0.001		
	100	14.64 ± 2.09	0.99	0.07 ± 0.005		
	110	20.92 ± 0.50	0.99	0.05 ± 0.002		

[a]Standard errors.

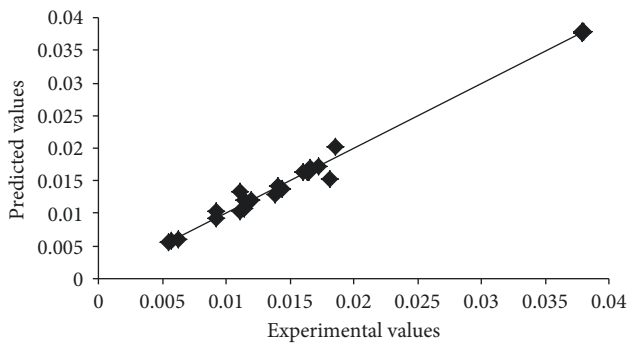

Figure 4: Correlation between the predicted and experimental C/C_0 values for PN simulated using (3).

of anthocyanins in PN, with an increase of k values from $8.21 \pm 3.49 \cdot 10^{-2}$ 1/min at 50°C to $20.92 \pm 0.50 \cdot 10^{-2}$ 1/min at 110°C can be observed (Table 2).

Wang and Xu [28] suggested significantly higher k values of $3.94 \cdot 10^3$ 1/min for the anthocyanin degradation in blackberry juice at 90°C. Harbourne [29] reported k value of 18.6 1/min for the degradation kinetic of anthocyanins in a blackcurrant model juice system at temperature of 100°C.

The $t_{1/2}$ values are expressed in (3) and presented in Table 2. The half-life at 50°C for PW, PCA, PS, PM, and PN were 6.27 ± 1.15 h, 25.08 ± 1.28 h, 3.13 ± 0.15 h, 10.03 ± 0.38, and 0.14 ± 0.003 h, respectively. A decrease in $t_{1/2}$ was found when the temperature increases, given values of 0.40 ± 0.02 h, 0.98 ± 0.15 h, 0.74 ± 0.09 h, 0.98 ± 0.04 h, and 0.05 ± 0.002 h at 120°C. From Table 2, it can be seen that the highest decrease in $t_{1/2}$ was determined for PW and PCA, being higher than that for the corresponding juices, whereas the most protective effect had glucose, with a decrease of only 76%. Harbourne [29] reported $t_{1/2}$ values of 2.18 ± 0.04 h at 100°C for the degradation kinetics of anthocyanins in a blackcurrant model juice system. Significantly higher values were found by Cemeroglu [30] for sour cherry juice of 54.34 and 8.1 h at 60°C and 80°C, which indicates that sour cherries anthocyanins are more heat stable than those in plum juices. The thermal degradation of anthocyanins in blood orange juice had $t_{1/2}$ values of 3.6 h at 80°C, as suggested by Kirca and Cemeroglu [31].

For the PN, the use of the fractional conversion kinetic model allowed the prediction of anthocyanins content and DPPH-RSA after prolonged heating at different temperatures (C_∞), which suggests that the final degree of degradation is temperature dependent (Figure 5).

The activation energy for anthocyanins thermal degradation in the simulated juices were 42.40 ± 6.87 kJ/mol in PW, 40.70 ± 4.25 kJ/mol in PCA, 23.03 ± 3.53 kJ/mol in PS, 35.99 ± 3.60 kJ/mol in PM, and 14.19 ± 2.39 kJ/mol in PN. The estimated E_a values are significantly lower than those reported in the literature, suggesting a significantly higher thermal stability of anthocyanins during heat processing of plum juices. For example, Danişman [32] reported E_a value

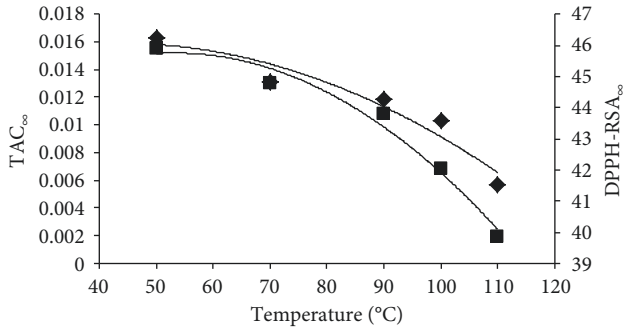

Figure 5: Correlations between TAC after prolonged heating time (TAC_{∞}) and the corresponding antioxidant activity (DPPH RSA_{∞}) in PN (■ DPPH-RSA, ◆ TAC).

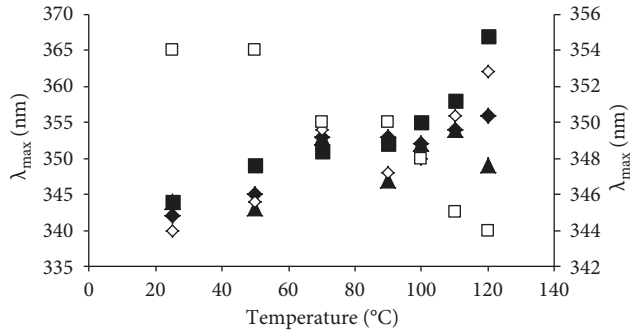

Figure 6: Heat-induced structural changes of anthocyanins from simulated and natural plum juices monitored as maximum emission wavelength (λ_{max}) at different temperatures when excited at 270 nm. PW (black diamonds), PCA (black squares), PS (black triangles), PM (empty diamonds), and PN (empty squares). Three independent tests were carried out in each case and SD was lower than 3.5%.

of 64.89 kJ/mol for anthocyanins degradation at the temperature range from 70 to 90°C in grape juice. Hillmann [33] reported E_a value of 72.74 kJ/mol for the thermal degradation of Bordo grape anthocyanins between 70°C and 90°C, whereas Wang and Xu [28] suggested 58.95 kJ/mol for the degradation of blackberry anthocyanins at temperatures ranging from 60°C to 90°C. A higher E_a value for anthocyanins thermal degradation in blood orange juice and concentrate was also reported by Kırca and Cemeroğlu [31], with values ranging from 73.2 to 89.5 kJ/mol as a function of solid content of studied system.

In our study, the estimated kinetic parameters indicate a greater temperature sensitivity of anthocyanins in PN. The degradation of anthocyanins in the presence of citric acid seems to be less susceptible to temperature increase than that of corresponding juices.

3.4. The Influence of Thermal Treatment on Fluorescence of Plum Juices. The absorption spectra of juices showed maxima at wavelength ranging from 270 to 410 nm (data not shown). Therefore, in order to evaluate the effect of heating of anthocyanins from juices, the samples were excited at different wavelengths such as 270 nm, 300 nm, 340 nm, and 410 nm. The most effective fluorescence intensities of juices were at the UV absorption maxima of 270 nm. Therefore, these spectra were further considered for the effect of heating on anthocyanins in plum juices. The short wavelength band in total fluorescence spectra, which covers the region of 270–330 nm in excitation and 295–360 nm in emission, is assigned to phenols [34]. It should be noted that forty-one phenolics were detected by Jaiswal [35] in plums, comprising caffeoylquinic acids, feruloylquinic acid, *p*-coumaroylquinic acids, methyl caffeoylquinates, methyl *p*-coumaroylquinate, caffeoyl-shikimic acids, catechin, epicatechin, rutin, esculin, quercetin, quercetin-3-O-hexosides, dimeric proanthocyanidins, trimeric proanthocyanidins, caffeoyl-glucoside, feruloyl-glucoside, p-coumaroyl-glucoside, vanillic acidglucosides, 3,4-dihydroxybenzoyl-glucoside, quercetin-3-O-pentosides, quercetin-3-O-rhamnoside, quercetin-pentoside-rhamnosides, and 3-*p*-methoxycinnamoylquinic acid, with chlorogenic acids and proanthocyanidins being found as the major compounds.

For PW, the spectra were dominated by emission band with maximum of 342 nm at 25°C, whereas red-shifts of 12–14 nm were observed by heating at temperatures of 110 and 120°C, respectively (Figure 6). In case of PCA, the maximum emission wavelength was found at 341 nm, whereas heating at 110°C caused a red shift of 17 nm and of 26 nm at 120°C. For PS, the spectra were characterized by emission bands with maximum at 344 nm, whereas heating caused a 7 nm red-shift at 90°C. Heating at higher temperature caused a 6 nm blue-shift at 100°C, followed by a small 3 nm red-shift at 120°C. Significant heat-induced structural changes were observed in case of PM. The fluorescence spectra at 25°C had the emission maximum at 340 nm. Heating at 70°C caused a significant 14 nm red-shift in λ_{max}. At temperatures ranging from 90 to 100°C, blue-shift of 6 nm and 4 nm were observed, followed by 6 nm and 12 nm red-shifts at temperatures of 110°C and 120°C, respectively. When exciting the PN at 270 nm, the emission spectra presented one band with maximum of 354 nm at 25°C. Heating caused structural changes characterized by blue-shifts ranging from 4 nm at 70°C–90°C to 9 nm at 110°C. Red- and blue-shifts in λ_{max} indicates sequential character of structural changes of anthocyanins induced by heat treatment.

The thermal degradation mechanism of anthocyanins was explained by Sadilova [27], involving the transition from the hemiketal to the chalcone form due to the increase of pH. Consequently, cyanidin/pelargonidin glycoside is transformed in chalcone glycoside due to the opening of the ring during the heat treatment, followed by deglycosylation, and the corresponding cleavage of the B- and A-ring with the formation of the protocatechuic acid/4-hydroxybenzoic acid and, respectively, phloroglucinaaldehyde. Four anthocyanin structures exist in equilibrium: flavylium cation, quinonoidal base, carbinol pseudobase, and chalcone. Nevertheless, it has been suggested that spectra with maximum at 350 nm obtained when excited at 260–270 nm are characteristic for hemiketal form of anthocyanins [36]. The fluorescence maximum at 350 nm are indicative of relatively simple and less-conjugated aromatic structural features, with the conjugated chromophores and electron-donating substituents (such as hydroxyl, methoxyl, and amino groups) contributing to the fluorescence in shorter wavelength regions. Hou [37] suggested that the stability of anthocyanins can increase with intermolecular

copigmentation. Plums contain mixtures of different compounds that may serve as copigments for intermolecular association with anthocyanins. A copigment may be one of flavonoids, alkaloids, amino acids, organic acids, nucleotides, polysaccarides, metals, and anthocyanins themselves [38]. In relation to the stability, anthocyanins may suffer reactions that altered their structures due to the electronic deficiency of their flavylium nuclei. Our results suggest that anthocyanins are unstable at high temperature, which caused a decrease in fluorescence intensity and red- and blue-shifts in maximum emission wavelengths.

4. Conclusions

In this study, the thermal stability of extracted anthocyanins from plums was examined in aqueous solutions, with or without the presence of different compounds, such as citric acids, glucose, and fructose, and a mixture of these substances. The degradation kinetics was compared with the thermal behavior of anthocyanins in natural juice. Four anthocyanins were identified in simulated and natural juices, namely, cyanidin 3-xyloside/cyanidin 3-glucoside, cyanidin 3-rutinoside, peonidin 3-glucoside and peonidin 3-rutinoside, whereas the real systems contained four more unidentified compounds. The heating at 100°C caused a significant decrease in anthocyanins content, with a protective effect found in simulated juices containing citric acid and sugars. Three anthocyanins were completely degraded in the real system, whereas in simulated juices containing citric acid and sugars, an increase in C3G and P3G was found.

In order to assess the effect of high temperature on total anthocyanin content in the simulated and natural plum juices, the first-order and fractional conversion kinetic models were used. The values reported for the activation energies highlighted that these bioactive compounds present a significant stability during thermal treatment. Fluorescence spectroscopy technique was used as an additional technique to evidence the heat treatment effects on the plum juices. The most effective fluorescence intensities of juices were at the UV absorption maxima of 270 nm. Heating caused significant red- and blue-shifts in emission maxima, suggesting important structural events.

Based on our results, further studies are currently developed by our research group for the determination of appropriate processing and formulation protocols that could lead to a more efficient utilization of plum anthocyanins as natural pigments in food products.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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