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ANTHOCYANINS, PHENOLS, AND ANTIOXIDANT ACTIVITY IN BLACKBERRY JUICE WITH PLANT EXTRACTS ADDITION DURING HEATING

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In this work the influence of addition of different plant extracts (olive leaf, green tea, pine bark PE 95%, pine bark PE 5:1, red wine PE 30%, red wine PE 4:1, and bioflavonoids) to blackberry juice during heating (at 30, 50, 70 and 90 °C) on the anthocyanin and phenol contents, polymeric colour, and antioxidant activity was investigated. Also, reaction rate constant, half-lives of degradation, and activation energy were calculated. Control sample was juice without addition of extracts. The highest anthocyanin content at 30 °C was in samples with the addition of olive leaf and green tea. At 90 °C the highest anthocyanin content was measured in samples with the addition of extract of red wine and bioflavonoides. Samples supplemented with the extracts had much higher antioxidant activity in comparison to the control sample. Results showed that at 90 °C the sample with green tea supplementation had the lowest reaction rate constant and the highest half-life. Activation energy ranged from 29 to 44 kJ mol^{-1}.

Keywords: anthocyanins, phenols, antioxidant activity, plant extracts, thermal degradation

Anthocyanins are bioactive compounds present in many fruit, vegetables, and their products. They are a sub-group of the flavonoids characterized by a C_6-C_3-C_6-skeleton. They are responsible for the characteristic colour of fruit and vegetables, thus playing a key role in influencing consumer sensory acceptance not only for fruit and vegetables, but also for their products (Patras et al., 2010).

The anthocyanin content in food products derived from fruit is much lower than in the raw material due to manufacturing and processing conditions, thus formulation of fruit products is very important. Thermal treatment is a necessary step for food preservation, thus, it is of high importance to evaluate the heat-induced anthocyanin degradation for establishing processes that are characterized by improved colour retention of products. Furthermore, anthocyanin degradation of products might serve as a marker of heat exposure (Sadilova et al., 2009). Fruit products should be manufactured by applying processing methods, conditions, and/or additives that would enhance not only the stability of anthocyanins but also the colour and higher nutritional value of food products, as well. During processing, temperature is one of the most important factors influencing anthocyanin stability. Thermal processing of foods involves heating temperatures from 50 to 150 °C, depending upon the pH of the product and the desired shelf life. The chemical stability of anthocyanins is the main focus of many recent studies due to their beneficial effects and their use as alternative to artificial colorants in foods (Patras et al., 2010).

The thermal degradation of anthocyanins was extensively studied (Gradinaru et al., 2003; Kirca et al., 2007; Harbourne et al., 2008) but there are only few studies on the prevention of thermal degradation of anthocyanins by the addition of different compounds,

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such as phenolic compounds (Gradinaru et al., 2003; Bąkowska et al., 2003; Kopjar et al., 2009a,b), sugars (Sadilova et al., 2009; Kopjar & Piližota, 2011), β-cyclodextrin (Mourtzinos et al., 2008), maltodextrin, gum arabic, a combination of maltodextrin and gum arabic, and soluble starch (Idham et al., 2012). It is also important to prevent thermal degradation of anthocyanins to avoid synthetic colorant application, since nowadays consumers are demanding less synthetic compounds in food products.

The objective of this study was to investigate the effect of addition of plant extracts (olive leaves, green tea, pine bark, red wine, bioflavonoids) on the anthocyanin content, total phenol content, and antioxidant activity during heating (at 30, 50, 70, and 90 °C) of blackberry juice.

1. Material and methods

1.1. Material

Blackberries were bought at the local market in Osijek (Croatia) and kept at –20 °C before sample preparation. Blackberry juice was prepared as follows: pressing the fruit through cheese cloth, centrifugation, and short heating at 90 °C for enzyme inactivation. Juice samples were prepared without and with the addition of selected crude extracts (0.1%), namely olive leaf (OL), green tea (GT), pine bark PE 95% (PB95), pine bark PE 5:1 (PB5:1), red wine PE 30% (RW30), red wine PE 4:1 (RW4:1), and bioflavonoids (B). Crude extracts were from Naturex (France) and obtained from Vitis (Croatia). The stabilization of samples was conducted in dark at 4 °C for 24 h before degradation studies were conducted.

1.2. Degradation studies

The thermal stability of anthocyanins in blackberry juice was studied at 30, 50, 70, and 90 °C. Aliquots of blackberry juice (20 ml) were put into well capped glass tubes to avoid the evaporation of samples. Tubes were put in a water bath preheated at the desired temperature. Samples were heated for 1 h at the desired temperature, removed from the water bath and rapidly cooled in ice-cooled water to room temperature. After cooling, evaluation of monomeric anthocyanins content, total phenol content, and antioxidant activity was carried out.

1.3. Monomeric anthocyanin content and polymeric colour determination

The monomeric anthocyanin pigment content of the samples was determined using the pH-differential method (Giusti & Wrolstad, 2001). Total monomeric anthocyanins were expressed as mg of cyanidin-3-glucoside per litre of sample (mg l^{-1}). Sample absorbance was read against a blank cell containing distilled water. The absorbance (A) of the sample was then calculated according to the following formula:

$$A=(A_{\lambda vis}-A_{700})_{pH\ 1.0}-(A_{\lambda vis}-A_{700})_{pH\ 4.5}. \quad (1)$$

The monomeric anthocyanin pigment content in the original sample was calculated according to the following formula:

$$\text{Anthocyanin content (mg } l^{-1})=(A\times MW\times DF\times 1000)/(\varepsilon\times l) \quad (2)$$

where DF was dilution factor, MW cyanidin-3-glucoside molecular weight (449.2), ε molar absorptivity (26 900), and l light path length.

Colour density, polymeric colour, and percent polymeric colour were determined using the bisulphite bleaching method as described by GIUSTI and WROLSTAD (2001). For the analysis, 0.2 ml of sodium bisulphite was added to 2.8 ml sample diluted with water, and 0.2 ml of water was added to 2.8 ml sample diluted with water. After equilibration for 15 min, samples were evaluated at $A_{\lambda vis}$, 700 nm and 420 nm.

Colour density was calculated using the control sample according to the following formula:

$$\text{Colour density}=[(A_{420}-A_{700})+(A_{\lambda vis}-A_{700})]\times DF. \quad (3)$$

Polymeric colour was determined using the bisulphite bleached sample using the following formula:

$$\text{Polymeric colour}=[(A_{420}-A_{700})+(A_{\lambda vis}-A_{700)}]\times DF. \quad (4)$$

Percent polymeric colour was calculated using the formula:

$$\%\ PC=(\text{polymeric colour/colour density})\times 100. \quad (5)$$

All measurements were conducted in duplicate.

1.4. Determination of total phenol content

The total phenol content was determined by the Folin-Ciocalteu method (OUGH & AMERINE, 1988). For the analysis, 0.2 ml of the sample was diluted in 1.8 ml of deionised water and 10 ml Folin-Ciocalteu reagent (1:10) was added and left to stand for 5 min. After that time 8 ml of a 7.5% Na_2CO_3 solution was added. After 2 h, absorbance was read at 765 nm using a spectrophotometer. Measurements were conducted in duplicate. Total phenol content was determined using a gallic acid calibration curve and results were expressed as g gallic acid equivalent per litre of sample (g l^{-1}).

1.5. Antioxidant activity

Antioxidant activity was determined using ABTS and DPPH methods. The ABTS assay followed the method of RE and co-workers (1999) with some modifications. Stock solutions of ABTS (7.4 mM) and potassium peroxodisulfate (2.6 mM) in water were prepared and mixed together. The mixture was left to react overnight (12–16 h) in the dark at room temperature. On the day of the analysis, the ABTS radical solution was diluted with ethanol to an absorbance of 1.136 at 734 nm. All measurements were performed as follows: 0.2 ml of sample was added to 2.8 ml of the ABTS radical solution. The mixture was left for 95 min and the absorbance was determined at 734 nm.

DPPH assay was conducted according to SHIMADA and co-workers (1992) with slight modifications; 0.2 ml of the sample was diluted with methanol, then 1 ml of DPPH solution (0.5 mM) was added. The mixture was shaken and left for 15 min at room temperature. Absorbance was measured at 517 nm using a spectrophotometer (Jenway 6300 Spectrophotometer).

Additional dilution was needed when the measured ABTS and DPPH value was over the linear range of the standard curve. For both methods measurements were conducted in

duplicate. The results were expressed as mmol trolox equivalents per 100 ml of sample (mmol/100 ml).

1.6. Calculation of kinetic parameters of anthocyanin degradation

The first-order reaction rate constants (k), half-lives ($t_{1/2}$), i.e. the time which is necessary for the degradation of 50% of anthocyanins, were calculated using the following equations:

$$\ln (c_t/c_0) = -k \times t \quad (6)$$

$$t_{1/2} = -\ln (0.5)/k \quad (7)$$

where c_0 is an initial anthocyanin content and c_t anthocyanin content after heating for a given time at the given temperature.

According to the Arrhenius equation, there is a linear relationship between lnk and 1/T:

$$k = k_0 \exp(-E_a/RT) \quad (8)$$

where E_a is the activation energy, R is gas constant, and T is temperature.

The Arrhenius activation energy (E_a) was calculated by plotting ln (k) against 1/T (absolute temperature in Kelvin).

1.7. Statistical analysis

The anthocyanin content was analysed by the analysis of variance (ANOVA) and Fisher's least significant difference (LSD) with significance defined at $P<0.05$. All statistical analyses were carried out using the software program STATISTICA 8 (StatSoft, Inc, USA). The results were expressed as means ± standard deviation.

2. Results and discussion

2.1. Stability of anthocyanins

Samples were heated at 30, 50, 70, and 90 °C for 1 h to determine the effect of plant extract addition on contents of anthocyanins and phenols and antioxidant activity. The results of anthocyanin content determination are presented in Table 1. Heating of samples at 30 °C caused slight decrease in the anthocyanin content, while at higher temperatures the decrease was to a greater extent. After the preparation of samples, the anthocyanin content of the control sample was 76.9 mg l^{-1}. Samples with the addition of pine bark (95%) had lower anthocyanin content (74.27 mg l^{-1}), while all other samples had higher anthocyanin contents (from 77.9 to 81.16 mg l^{-1}) compared to control. Kopjar and co-workers (2011) investigated the influence of supplementation with the same plant extracts on the anthocyanin content of blackberry juice during storage. They found that samples with the addition of plant extracts had higher anthocyanin contents. Co-pigmentation effect was the most likely reason for this increase of anthocyanin content. Phenolic compounds act as co-pigments and through co-pigmentation effect the anthocyanin stability improves. The attack by water converts the flavylium ion to colourless pseudobase resulting in colour loss. Several factors influence co-pigmentation, among which co-pigment type is the most important (Mazza & Brouillard,

1990; BĄKOWSKA et al., 2003). Blackberry juice without addition of extracts had its absorbance maximum at 510 nm, while samples with addition of extracts had absorbance maximums at higher λ. The difference in the increase of λ (Δλ), i.e. bathochromic shift, for different extracts was different. Δλ were 3 nm for pine bark PE 5:1 and bioflavonoides, and 2 nm for all other extracts (KOPJAR et al., 2011).

Table 1. Anthocyanin content (mg l^{-1}) after 1 h heating at 30, 50, 70, and 90 °C of blackberry juice without and with plant extract addition

Samples	"0"	30 °C	50 °C	70 °C	90 °C
BJ	76.90±0.11^{a}	73.77±0.13^{a,e}	68.76±0.15^{a}	53.35±0.16^{a}	45.21±0.14^{a}
BJ+OL	78.65±0.12^{b}	75.77±0.14^{b}	69.38±0.18^{b}	53.48±0.12^{a}	45.01±0.16^{a}
BJ+PB95	74.27±0.18^{c}	71.39±0.15^{c}	65.38±0.13^{c}	50.72±0.18^{b}	42.03±0.11^{b}
BJ+GT	78.03±0.21^{b}	75.52±0.16^{b}	70.89±0.19^{d}	55.73±0.14^{c}	46.05±0.16^{c}
BJ+PB5:1	77.90±0.20^{b}	71.13±0.11^{c}	67.85±014^{e}	53.48±0.16^{a}	44.92±0.14^{a}
BJ+RW30	79.53±0.10^{d}	72.01±0.19^{d}	65.75±0.12^{c}	54.23±0.13^{d}	46.85±0.15^{d}
BJ+RW4:1	80.66±0.29^{e}	73.52±0.13^{a}	70.26±0.20^{d}	54.35±0.16^{d}	47.01±0.12^{d}
BJ+B	81.16±0.25^{e}	74.14±0.11^{e}	70.64±0.13^{d}	55.61±0.19^{c}	46.99±0.10^{d}

BJ: blackberry juice; OL: olive leaves; PB95: pine bark 95%; GT: green tea; PB5:1: pine bark 5:1; RW30: red wine 30%; RW4:1: red wine 4:1; B: bioflavonoids. Values in the same column with different superscripts (a-e) are significantly different ($P<0.05$) by analysis of variance (ANOVA) and Fisher's least significant difference (LSD)

During heating degradation of anthocyanins occurred. The anthocyanin content ranged from 42.03 mg l^{-1} to 75.77 mg l^{-1} depending on the heating temperature and the added plant extract. Different tendencies were observed for the decrease of anthocyanin content at different temperatures, showing the importance of the type of plant extract and probably its chemical composition. The anthocyanin content of the control sample was 73.77 mg l^{-1}, 68.76 mg l^{-1}, 53.35 mg l^{-1}, and 45.21 mg l^{-1} at 30, 50, 70, and 90 °C, respectively. At 30 °C higher anthocyanin content was measured in the control sample, than in samples with addition of olive leaves and green tea extracts (75.77 mg l^{-1} and 75.52 mg l^{-1}, respectively). At 50 °C samples with addition of olive leaves, green tea, red wine 4:1, and bioflavonoid extracts had higher anthocyanin content (68.38 mg l^{-1}, 70.89 mg l^{-1}, 70.26 mg l^{-1}, and 70.64 mg l^{-1}, respectively) in comparison to the control sample. At 70 °C and 90 °C the same tendency was observed. Samples with the addition of green tea, red wine 30%, red wine 4:1 and bioflavonoid extracts had higher anthocyanin content compared to the control sample. Protection of the flavylium ring against attack from water is absolutely necessary to maintain the intensity of coloured solutions. One way of retaining anthocyanin colour is by removal of water and displacement of the hydration/dehydration equilibrium towards the coloured species (i.e. reduce the extent of the hydration reaction) (BROUILLARD, 1983). Two mechanisms were suggested to be responsible for thermal degradation of anthocyanins: hydrolysis of the 3-glycoside linkage to form the more labile aglycone and hydrolytic opening of the pyrilium ring to form a substituted chalcone, which then degrades to a brown insoluble compound of polyphenolic nature (SIMPSON, 1985). Probably, some plant extracts through co-pigmentation effect and/or suppression of one of these two mechanisms were responsible for the decrease of thermal degradation of anthocyanins. Red currant juice samples with addition of phenolic

compounds (catechol, 4-methyl catechol, gallic acid, catechin, and chlorogenic acid) had higher anthocyanin content in comparison to red currant juice, showing that phenolic compounds prevented the thermal degradation of anthocyanins (Kopjar et al., 2009). Dyrby and co-workers (2001) reported greater thermal stability of anthocyanins present in red cabbage compared to blackcurrant, and of grape skin and elderberry anthocyanins in soft drink model system due to the protection of flavylium system through co-pigmentation.

Thermal degradation of blackberry anthocyanins was also evaluated through calculation of reaction rate constants and half-lives. Assuming that degradation of anthocyanins fits first-order reaction model, as it was proven in many studies earlier (Gradinaru et al., 2003; Kirca et al., 2007; Domínguez-López et al., 2008; Yue & Xu, 2008; Kopjar et al., 2011), it was possible to calculate the reaction rate constants and the half-life of anthocyanin degradation. Calculated kinetic parameters are presented in Table 2. Results of reaction rate constants ranged from 0.032 h^{-1} to 0.569 h^{-1}, while results of half-life of anthocyanins ranged from 1.217 h to 21.199 h, both depending on temperature and type of extract. With the increase of heating temperature reaction rate constant increased, while half-life of anthocyanins decreased. The sample with the addition of green tea extract had the lowest reaction rate constants and the highest half-life of anthocyanins. Kopjar and co-workers (2009) showed that all samples with addition of phenolic compounds (catechol, 4-methyl catechol, gallic acid, catechin, and chlorogenic acid) were more stable to heat than control samples. All samples with phenolic compound had lower reaction rate constants and higher half-life of anthocyanins.

The activation energy is the energy needed by a system to initiate a particular process. Activation energy (Table 2) was calculated from Arrhenius plot, since the correlation between ln k and 1/T was very high (0.952 to 0.988). Activation energy for control sample was 40.64 kJ mol^{-1}, while samples with addition of olive leaves, pine bark 95%, and green tea (42.65 kJ mol^{-1}, 41.96 kJ mol^{-1}, and 44.16 kJ mol^{-1}, respectively) had higher activation energy, meaning that it was necessary to bring higher amount of energy to reaction system so that degradation of anthocyanins would occur. All other samples had lower activation energy (26.29 kJ mol^{-1} to 29.26 kJ mol^{-1}). Mourtzinos and co-workers (2008) observed that sample without and with β-cyclodextrin had similar values of activation energy, 54.05 kJ mol^{-1} and 54.02 kJ mol^{-1}, thus concluding that degradation mechanism of anthocyanins inside the β-cyclodextrin and of the free anthocyanins were the same. In our case, activation energies were higher or lower when extracts were added in comparison to control sample, probably due to different mechanism of anthocyanin degradation. Study of Kopjar and co-workers (2009b) showed that red currant juice anthocyanins had much lower activation energy (33 kJ mol^{-1}) than samples with addition of phenolic compounds (35 kJ mol^{-1} to 45 kJ mol^{-1}). Anthocyanin stability during heating highly depends on initial anthocyanin content, matrix composition, pH, and solid content (Bąkowska et al., 2003; Gradinaru et al., 2003; Kirca et al., 2007; Mourtzinos et al., 2008; Kopjar et al., 2009a; Kopjar & Piližota, 2011).

Percentage results of polymeric colour determination are presented in Table 3. After preparation of samples, the control sample had a polymeric colour value of 31.81%. Only the sample with addition of green tea extract had lower polymeric colour, 30.01%, while all other samples had higher values (32.16% to 37.82%). During heating polymeric colour percentage increased, indicating that formation of polymeric complexes occurred. Decrease of anthocyanin content in samples was followed by increase of polymeric colour. The high temperatures (blanching at 95 °C for 3 min in combination with pasteurization) involved in the processing of blueberries into purees resulted in 43% loss in total monomeric anthocyanins,

compared to original levels found in fresh fruit, whereas polymeric colour values increased from 1% to 12% (Brownmiller et al., 2008).

Table 2. Kinetic parameters after 1 h heating at 30, 50, 70, and 90 °C of blackberry juice without and with plant extract addition

Samples	T (°C)	k (h^{-1})	$t_{1/2}$ (h)	E_a (kJ mol^{-1})
BJ	30	0.042	16.680	40.64
	50	0.111	6.195	(0.976)*
	70	0.365	1.895	
	90	0.531	1.304	
BJ+OL	30	0.037	18.580	42.65
	50	0.125	5.527	(0.973)
	70	0.385	1.797	
	90	0.558	1.241	
BJ+PB95	30	0.039	17.521	41.96
	50	0.127	5.436	(0.977)
	70	0.381	1.817	
	90	0.569	1.217	
BJ+GT	30	0.032	21.199	44.16
	50	0.095	7.222	(0.981)
	70	0.336	2.059	
	90	0.527	1.314	
BJ+PB5:1	30	0.090	7.623	29.26
	50	0.138	5.018	(0.963)
	70	0.376	1.842	
	90	0.550	1.259	
BJ+RW30	30	0.099	6.978	26.29
	50	0.190	3.642	(0.988)
	70	0.382	1.810	
	90	0.529	1.309	
BJ+RW4:1	30	0.092	7.478	28.97
	50	0.138	5.021	(0.952)
	70	0.394	1.755	
	90	0.539	1.283	
BJ+B	30	0.090	7.661	29.23
	50	0.138	4.992	(0.963)
	70	0.378	1.833	
	90	0.546	1.268	

BJ: blackberry juice; OL: olive leaves; PB95: pine bark 95%; GT: green tea; PB5:1: pine bark 5:1; RW30: red wine 30%; RW4:1: red wine 4:1; B: bioflavonoids. *Number in parentheses presents correlation coefficient of relation between ln k and 1/T

Table 3. Polymeric colour (%) after 1 h heating at 30, 50, 70, and 90 °C of blackberry juice without and with plant extract addition

Samples	"0"	30 °C	50 °C	70 °C	90 °C
BJ	31.81±0.21^{a}	37.96±0.11^{a}	44.76±0.21^{a}	53.14±0.12^{a}	61.91±0.25^{a}
BJ+OL	32.16±0.14^{b}	34.58±0.12^{b}	39.92±0.23^{b}	42.04±0.22^{b}	54.76±0.31^{b}
BJ+PB95	32.57±0.31^{b,c}	41.40±0.21^{c}	48.54±0.31^{c}	54.69±0.19^{c}	61.20±0.24^{a}
BJ+GT	30.01±0.21^{a}	36.25±0.23^{d}	41.94±0.32^{d}	49.69±0.21^{d}	55.36±0.34^{b}
BJ+PB5:1	37.82±0.19^{e}	45.02±0.17^{e}	52.21±0.12^{e}	59.63±0.12^{e}	65.12±0.15^{c}
BJ+RW30	34.55±0.25^{d}	39.11±0.19^{f}	44.96±0.23^{a}	54.50±0.18^{c}	59.12±0.21^{d}
BJ+RW4:1	33.19±0.11^{c}	35.53±0.25^{d}	42.47±0.30^{d}	49.36±0.23^{d}	56.32±0.12^{e}
BJ+B	35.09±0.29^{d}	42.12±0.32^{c}	49.25±0.32^{c}	56.32±0.17^{e}	61.25±0.26^{a}

BJ: blackberry juice; OL: olive leaves; PB95: pine bark 95%; GT: green tea; PB5:1: pine bark 5:1; RW30: red wine 30%; RW4:1: red wine 4:1; B: bioflavonoids. Values in the same column with different superscripts (a-f) are significantly different (P<0.05) by analysis of variance (ANOVA) and Fisher's least significant difference (LSD)

2.2. Total phenol content and antioxidant activity

Total phenol contents in samples with addition of plant extracts were higher (Table 4) compared to control. That was expected, since investigated plant extracts are rich in phenolic compounds. Total phenol content and antioxidant activity of water solution of selected crude extracts were presented by Kopjar and co-workers (2009a). Total phenol content of control sample was 0.31 g l^{-1}. The highest total phenol content was measured for samples with addition of pine bark 95% and green tea (~0.91 g l^{-1}), while other samples contained less phenols (0.53 g l^{-1} to 0.66 g l^{-1}). During heating degradation of phenols occurred, samples with addition of pine bark 95% and green tea retained the highest total phenol content. Samples with addition of plant extracts also had higher antioxidant activity determined with DPPH and ABTS method (Tables 5 and 6). During heating antioxidant activity increased.

Table 4. Total phenol content (g l^{-1}) after 1 h heating at 30, 50, 70, and 90 °C of blackberry juice without and with plant extract addition

Samples	"0"	30 °C	50 °C	70 °C	90 °C
	0.31±0.01^{a}	0.29±0.02^{a}	0.25±0.03^{a}	0.20±0.03^{a}	0.18±0.01^{a}
BJ+OL	0.53±0.02^{b}	0.48±0.01^{b}	0.45±0.02^{b}	0.41±0.01^{b}	0.39±0.01^{b,f}
BJ+PB95	0.91±0.04^{c}	0.89±0.04^{c}	0.87±0.01^{c}	0.84±0.02^{c}	0.81±0.02^{c}
BJ+GT	0.90±0.03^{c}	0.87±0.03^{c}	0.84±0.02^{d}	0.82±0.02^{c}	0.79±0.02^{c}
BJ+PB5:1	0.59±0.02^{d}	0.55±0.05^{d}	0.52±0.03^{e}	0.49±0.01^{d}	0.46±0.01^{d}
BJ+RW30	0.66±0.06^{e}	0.62±0.01^{e}	0.59±0.04^{f}	0.56±0.03^{e}	0.52±0.02^{e}
BJ+RW4:1	0.51±0.04^{f}	0.48±0.02^{b}	0.45±0.01^{b}	0.42±0.01^{b}	0.38±0.02^{b}
BJ+B	0.53±0.01^{b}	0.50±0.02^{b}	0.47±0.01^{g}	0.45±0.01^{f}	0.41±0.01^{f}

BJ: blackberry juice; OL: olive leaves; PB95: pine bark 95%; GT: green tea; PB5:1: pine bark 5:1; RW30: red wine 30%; RW4:1: red wine 4:1; B: bioflavonoids. Values in the same column with different superscripts (a-g) are significantly different (P<0.05) by analysis of variance (ANOVA) and Fisher's least significant difference (LSD).

Table 5. Antioxidant activities determined by ABTS method (mmol/100 ml) after 1 h heating at 30, 50, 70, and 90 °C of blackberry juice without and with plant extract addition

Samples	"0"	30 °C	50 °C	70 °C	90 °C
BJ	0.42±0.012^{a}	0.50±0.014^{a}	0.65±0.014^{a}	0.78±0.011^{a}	0.78±0.015^{a}
BJ+OL	0.81±0.013^{b}	0.90±0.011^{b}	0.92±0.015^{b}	0.97±0.010^{b}	1.03±0.011^{b}
BJ+PB95	1.01±0.010^{c}	1.14±0.009^{c}	1.35±0.011^{c}	1.62±0.010^{c,d}	1.98±0.010^{c}
BJ+GT	1.11±0.010^{d}	1.30±0.010^{d}	1.45±0.013^{d}	1.59±0.014^{d}	1.78±0.012^{d}
BJ+PB5:1	1.02±0.011^{c}	1.21±0.012^{e}	1.45±0.012^{d}	1.63±0.010^{c}	1.82±0.012^{e}
BJ+RW30	1.08±0.009^{e}	1.11±0.008^{f}	1.35±0.016^{c}	1.52±0.011^{e}	1.79±0.010^{d,e}
BJ+RW4:1	0.99±0.014^{c}	1.03±0.010^{g}	1.32±0.018^{c}	1.59±0.010^{d}	1.81±0.011^{e}
BJ+B	1.09±0.008^{d,e}	1.21±0.013^{e}	1.45±0.011^{d}	1.69±0.10^{f}	1.92±0.009^{f}

BJ: blackberry juice; OL: olive leaves; PB95: pine bark 95%; GT: green tea; PB5:1: pine bark 5:1; RW30: red wine 30%; RW4:1: red wine 4:1; B: bioflavonoids. Values in the same column with different superscripts (a-g) are significantly different ($P<0.05$) by analysis of variance (ANOVA) and Fisher's least significant difference (LSD)

Table 6. Antioxidant activities determined by DPPH method (mmol/100 ml) after 1 h heating at 30, 50, 70, and 90 °C of blackberry juice without and with plant extract addition

Samples	"0"	30 °C	50 °C	70 °C	90 °C
BJ	0.58±0.011^{a}	0.65±0.015^{a}	0.87±0.012^{a}	0.96±0.012^{a}	1.02±0.016^{a}
BJ+OL	1.15±0.010^{b}	1.26±0.010^{b}	1.45±0.010^{b}	1.69±0.010^{b}	1.89±0.011^{b}
BJ+PB95	1.38±0.012^{c}	1.42±0.011^{c}	1.56±0.010^{c}	1.70±0.011^{b}	1.92±0.012^{c}
BJ+GT	1.31±0.009^{d}	1.43±0.010^{c}	1.52±0.012^{d}	1.87±0.012^{c}	1.93±0.011^{c}
BJ+PB5:1	1.25±0.011^{e}	1.36±0.015^{d}	1.52±0.011^{d}	1.78±0.011^{d}	1.89±0.012^{b}
BJ+RW30	1.29±0.012^{d,e}	1.41±0.015^{c}	1.62±0.011^{e}	1.82±0.010^{e}	1.96±0.013^{d,e}
BJ+RW4:1	1.05±0.011^{b}	1.21±0.011^{e}	1.52±0.012^{d}	1.78±0.012^{d}	1.94±0.012^{c,d}
BJ+B	1.20±0.010^{f}	1.32±0.018^{d}	1.52±0.012^{d}	1.87±0.010^{c}	1.99±0.014^{e}

BJ: blackberry juice; OL: olive leaves; PB95: pine bark 95%; GT: green tea; PB5:1: pine bark 5:1; RW30: red wine 30%; RW4:1: red wine 4:1; B: bioflavonoids. Values in the same column with different superscripts (a-f) are significantly different ($P<0.05$) by analysis of variance (ANOVA) and Fisher's least significant difference (LSD)

Thermal treatment of samples causes degradation of nutrients, but different chemical reaction can also occur within the matrix. Chemical oxidation of phenols can also lead to loss of antioxidant activity, but it was found that partially oxidised phenols can demonstrate higher antioxidant activity than unoxidised phenols (Manzocco et al., 2001). Thermal degradation of anthocyanins results in the formation of polyphenolic degradation products. It is not clear whether the formation of these components results in an overall reduction in antioxidant activity, because the polyphenolic components formed may also possess antioxidant properties (Patras et al., 2010). In our case, probably degradation products of anthocyanins and partially oxidised phenols are responsible for the increased antioxidant activity. Correlation between antioxidant activity and phenol content is lower at higher temperatures (0.3380–0.4267), meaning that antioxidant activity does not depend only on the phenol content, but also on other products formed through the degradation reactions. There are some

differences in values obtained by different methods for antioxidant activity due to various responses of various phenolic compounds in different applied methods (different free radicals were used) (Singleton & Rossi, 1965).

3. Conclusions

Temperature is one of the most important factors that influence the stability of anthocyanins. By preventing thermal degradation of anthocyanins, the colour of food product is also retained and there is no need for application of synthetic colorants. In this article, the influence of plant extracts on the anthocyanin content in blackberry juice during heating was investigated. Addition of different plant extracts could be a possible mean for improving anthocyanin stability. Also, increase of phenol and antioxidant content would be achieved, compounds that are known to have beneficial effect on human health.

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