

PROFESSIONAL PAPER

Colour stability and antioxidant activity of some berry extracts

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Abstracts

The colour stability of the blackberry, mulberry and blueberry extracts by monitoring the changes in colour parameters and remaining absorbance as a result of increased temperature and heating time were examined. The knowledge of colour stability is important for optimization of production and storage of coloured food products. The aim of present study was also to determine and compare the total phenols content and antioxidant activity of these dark coloured berry fruits. The results showed that highest total phenols content was determined in blueberry extracts (3198.50 mg kg⁻¹). Extracts were analyzed for antioxidant activity by the ABTS and DPPH methods. The heating time and temperature affected the colour stability of the berry fruit extracts. The maximum colour stability was determined for mulberry extract.

Keywords: blackberry, mulberry, blueberry, colour, heating

1.0. Introduction

Berry fruits are considered as a functional food which is consumed as part of the usual diet and may help to promote optimal health and reduce the risk of chronic diseases beyond basic nutrition. Most of phenolic compounds of berry fruits are responsible for the colour and flavor of the fruit and have significant antioxidant capacity (Rios de Souza et al, 2014). Blackberry, mulberry and blueberry fruits are abundant in anthocyanins (Kaume et al, 2012; Paredes-Lopez et al., 2010). Anthocyanins are water-soluble plant secondary metabolites consisting of one or more aromatic rings with different degrees of hydroxylation, methoxylation and glycosylation, contributing to fruit colour and bitterness. Anthocyanins are of great interest for the food industry because they provide a wide range of colours (red, orange, violet and blue colours) in many flowers, vegetables and fruits and can be used as food colourant from natural sources as an promising alternative to synthetic colourants. The food trend towards natural products stimulated the interest of industry producers of food products for use of natural pigments in foodstuffs. However, due to its low stability depending on the process conditions during processing and storage, the introduction of these compounds in food is a great challenge (Hui et al., 2006; Lobo et al., 2010).

The main objective of present study was to determine the stability of the colour of berry extracts by monitoring the changes in colour parameters and absorbance as a result of increased temperature and heating time. Furthermore, the aim of this study was to determine antioxidant activity as well as to evaluate the amount of phenolics of three different berry fruits (mulberry, blackberry and blueberry).

2.0. Materials and methods

2.1. Fruit samples

Samples of berry fruits: blackberry (*Rubus fruticosus*), mulberry (*Morus sp.*) and blueberry (*Vaccinium Myrtilus L.*)

were purchased on local market (Zagreb, Croatia). Samples were frozen and stored at -40°C in plastic bags for subsequent analysis.

2.2. Preparation of berry extracts

Extraction of phenolic compounds was carried out using 10 g of each fruit, 20 mL of 30% (v/v) aqueous ethanol and 2 mL 0.1% HCl. This mixture was sonicated in ultrasonic bath (Elmasonic P 70H, Elma, Siegen, Germany) for 30 min at 70°C and filtered through Whatman filter paper No. 40 (Kent, UK). The obtained extracts were used for determination of total phenol and anthocyanin content, the content of anthocyanins and phenolic compounds by high performance liquid chromatography (HPLC) method, antioxidant activity by DPPH method and antioxidant capacity by ABTS method. Extracts were stored at -20 °C until analysis.

2.3. Determination of total phenol content

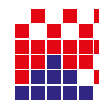
The total phenol content (TPC) of berry extracts was determined spectrophotometrically using Folin-Ciocalteu reagent (Ough and Amerine, 1998). Results were expressed as mg of gallic acid equivalents per 1000 g of sample (mg GAE/kg).

2.4. Determination of monomeric anthocyanins

The total monomeric anthocyanin content was determined according to the pH differential method (AOAC, 2006). This method is based on the anthocyanin structural transformation that occurs with a change in pH (coloured at pH 1.0 and colourless at pH 4.5). The final concentration of monomeric anthocyanins was calculated as equivalents of cyanidin-3-glucoside (mg L⁻¹).

2.5. Determination of antioxidant activity and capacity

The free radical scavenging activity of berry extracts was evaluated using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay. DPPH method is based on the measurement of the colour loss of DPPH solution by the change of absorbance of 517



nm caused by the reaction of DPPH with the tested sample (Prakash, 2001). The total antioxidant activity was calculated according to equation:

$$S = 100 - \left(\frac{A_x}{A_0} \times 100 \right)$$

where: S – antioxidant activity (%); A_x – sample absorbance; A_0 – control sample absorbance

The radical scavenging capacity of berry extracts was evaluated by the radical cation decolourization assay as described by Re et al. (1999) and Pellegrini et al. (1999). This method measures the relative ability of various antioxidant molecules to scavenge and decolourize the free [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation (ABTS^{•+}), a blue-green chromophore with characteristic absorption at 734 nm. Antioxidant molecule reduce radical cation (ABTS^{•+}) to ABTS. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; SigmaAldrich) was used as antioxidant standard.

2.6. Determination of the extract colour

Measurement of extracts colour was performed using a colorimeter (CM-3500d, Konica Minolta, Japan). Data were analyzed using SpectraMagic NX software by CIELAB colour system. Colour parameters of extracts are shown as L^* , a^* , b^* , C^* , H^* parameters.

2.7. Determination of colour stability of extracts

Colour degradation of the berry extracts was determined during heating at 70 °C according to the method described by Fernandez-Lopez et al. (2013). Briefly, blackberry, mulberry and blueberry extracts were diluted with distilled water to make their final absorbance 0.700 ± 0.005 at 520 nm. Prepared diluted extracts were stored in the dark at a temperature of 4°C overnight. The diluted extracts were thermostated in a water bath at 70 °C. The changes in absorbance were monitored between 400 and 700 nm at intervals of 15, 30, 45, 60, 120 and 180 minutes as well as changes in colour parameters L^* , a^* , b^* , C^* and h^* . At this specified time intervals sample extracts are excluded and immediately chilled in an ice bath to stop further colour thermal degradation. The total colour change of extracts relative to the control sample is calculated according to the Euclidean distance between the colour parameters L , a and b where ΔE means the deviates of extract colour from reference colour.

2.8. Statistical analysis

Statistical analyzes were performed using the SAS® version 9.3. Data were subjected to the one-way analysis of variance (ANOVA) for comparison of colour change in thermal treated samples. Mean values were compared by t test (LSD), and they are considered significantly different at $p \leq 0.001$.

3.0. Results and discussion

Total phenol content (mg kg^{-1}) of the examined samples of blackberry, mulberry and blueberry fruits is presented in Fig. 1. Significant difference in total phenol content were determined. Total phenol content ranged from $2276.00 \text{ mg kg}^{-1}$ (mulberry) to $3198.50 \text{ mg kg}^{-1}$ (blueberry) and these results are in agreement with other published literature data (Becker Pertu-

zatti et al., 2014; Shen et al., 2014; Contessa et al., 2013; Kara and Erçelebi, 2013; Ercisli and Orhan, 2007). Several studies have suggested that differences in total phenol content could be explained by genetic factors (species, variety, cultivation), environmental conditions (region, organic farming, growing season, weather conditions), maturity stage and intra-fruit variation, postharvest management (storage time and conditions: temperature, controlled and/or modified atmospheres, edible coatings) as well as processing conditions (temperature, pH value, metal ions, exposure to light, exposure to oxygen and enzymatic activities (Szajdek and Borowska, 2008; Bakowska et al., 2003; Šavikin et al., 2009; Jimenez-Garcia et al., 2013; Manganaris et al., 2014; Cantin et al., 2012).

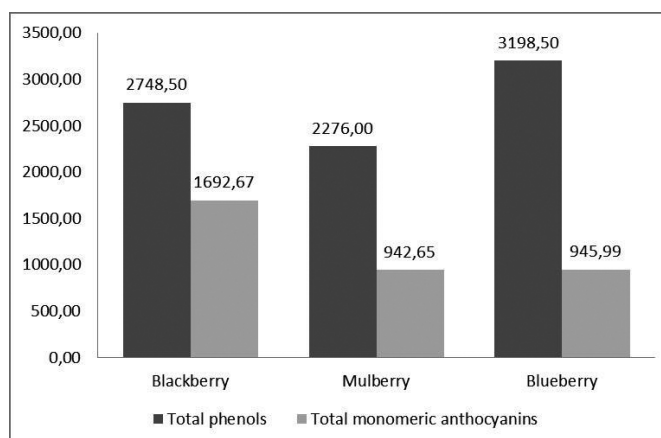


Figure 1. Total phenol and total monomeric anthocyanin content (mg kg^{-1})

Content of monomeric anthocyanins determined using the pH differential method, was significantly higher in the blackberry extract than in mulberry and blueberry extract (Fig. 1). Furthermore, anthocyanin content of analyzed extracts in this research are higher than in recently reported literature data (Rios de Souza et al, 2014, Contessa et al., 2013) which indicates high nutritional quality of examined blackberry, mulberry and blueberry fruits. Several studies have suggested different results of anthocyanin content and type in the blackberry which mainly depends on geographical origin and maturation stage (Bowen-Forbes et al., 2010; Shahidi and Naczka, 2004). Nutritional epidemiological data show that bioactive compounds have potential antioxidant, antihyperlipidemic, antihyperglycemic, antihypertensive, antimicrobial, anti-inflammatory and antineurodegenerative properties, both in vitro and in vivo (Paredes-Lopez et al., 2010; Shen et al., 2014; Gry et al., 2007; Stefanut et al., 2013). Generally, antioxidant activity is in the correlation with the content of bioactive compounds such as: vitamins, phenols, anthocyanins while differences can be caused by many agronomic and technological factors like differences in cultivars, method of cultivation, climatological conditions, ripening stage, harvest, post-harvest treatments, storage and processing conditions (Jakobek et al., 2009; Moyer et al., 2002). Antioxidant activity of blackberry, mulberry and blueberry extracts was evaluated using two common methods (DPPH and ABTS). The highest antioxidant activity achieved by DPPH method was observed for blueberry 53.58% followed by blackberry and mulberry. (Fig. 2a, 2b). The same trends of antioxidant activity were achieved by ABTS method

Table 1. Colour parameters of blackberry, mulberry and blueberry extracts

Extract	L*	a*	b*	C*	H*
	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
Blackberry	76.74 ^a	37.23 ^a	11.59 ^b	39.18 ^a	17.31 ^b
Mulberry	61.83 ^b	24.96 ^b	14.22 ^a	28.73 ^b	29.67 ^a
Blueberry	73.94 ^a	38.17 ^a	3.84 ^c	38.36 ^a	5.75 ^c

L* = lightness; a* = red-green values; b* = blue-yellow values; C* = chroma; H* = angle; different letters indicate significant differences between means at $p < 0.001$

which confirmed the highest antioxidant activity for blueberry (304.95 $\mu\text{mol Trolox L}^{-1}$) followed by blackberry and mulberry. The antioxidant activity of berry fruit is highly correlated with its content of phenolic compounds (Pantelidid et al., 2007). The same tendency was observed in this study.

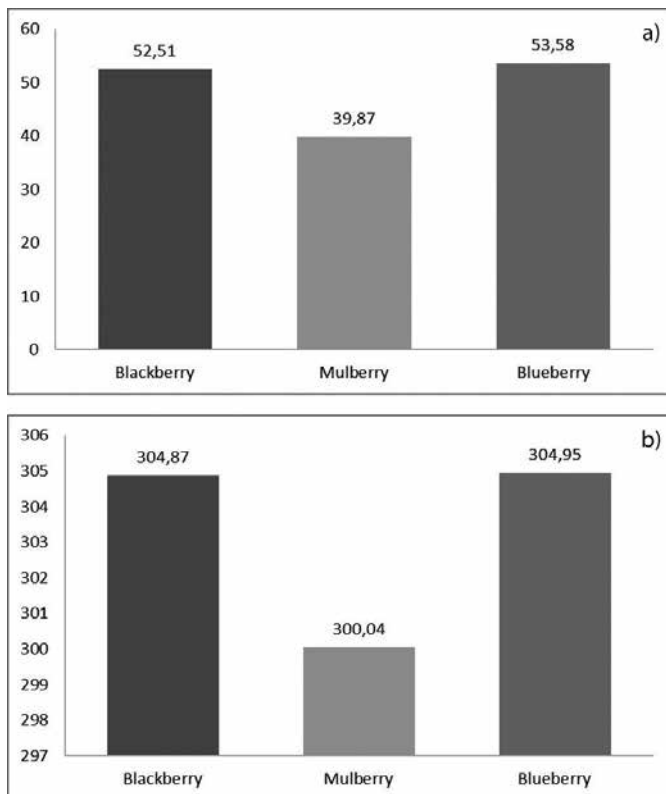


Figure 2. Antioxidant activity (%) of blackberry, mulberry and blueberry extracts by DPPH method (a) and ABTS method (b)

Blackberry, mulberry and blueberry are rich in anthocyanins which are characteristic dark red or blue and in food industry have a great potential for use as natural colourants. The colour degradation of blackberry, mulberry and blueberry extracts after thermal processing was measured by lightness (L*), redness (a*) and yellowness (b*), total colour difference (ΔE) and are shown in Table 1.

Significant differences ($p < 0.001$) in all analyzed colour parameters were determined. L* parameter indicates the intensity of light or darkness. The highest L* value is recorded for blackberry extract followed by blueberry extract suggesting that the extracts of blackberry and blueberry are brighter compared to the extract of mulberry. The a* parameter indicates the intensity of the red or green colour. Blackberry and blueberry extracts have a higher proportion of red component than mul-

berry extract. The mulberry extract have the highest recorded value of b* parameter indicating the presence of blue colour (Tables 2-4).

Table 2. The changes of colour parameters in blackberry extract at 70°C/180 min

Time (min)	L*	a*	b*	C*	h*	ΔL	Δa	Δb	ΔE
	***	***	***	***	***	***	***	***	***
15	25.58 ^d	12.41 ^d	3.87 ^b	13.00 ^d	5.77 ^b	-51.16 ^a	-24.82 ^a	-7.73 ^a	57.39 ^a
30	34.11 ^c	16.55 ^c	5.16 ^c	17.33 ^c	7.69 ^b	-42.63 ^b	-20.68 ^b	-6.44 ^b	47.82 ^b
45	45.48 ^a	22.06 ^a	6.87 ^a	23.11 ^a	10.26 ^a	-31.26 ^d	-15.17 ^d	-4.73 ^c	35.07 ^f
60	35.05 ^c	17.01 ^c	5.30 ^{bc}	17.81 ^c	7.91 ^b	-41.69 ^b	-20.22 ^b	-6.30 ^b	46.76 ^c
120	38.21 ^b	18.54 ^b	5.78 ^{bc}	19.42 ^b	8.62 ^{ab}	-38.53 ^c	-18.69 ^c	-5.82 ^b	43.22 ^d
180	39.58 ^b	19.20 ^b	5.98 ^b	20.12 ^b	8.93 ^{ab}	-37.16 ^c	-18.03 ^c	-5.62 ^{bc}	41.68 ^e

L* = lightness; a* = red-green values; b* = blue-yellow values; C* = chroma; h* = angle; ΔL , Δa , Δb = changes after heating; ΔE = total color change; different letters indicate significant differences between means at *** $p < 0.001$

Table 3. The changes of colour parameters in mulberry extract at 70°C/180 min

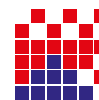
Time (min)	L*	a*	b*	C*	h*	ΔL	Δa	Δb	ΔE
	***	***	**	***	***	***	***	***	***
15	24.65 ^e	12.72 ^d	1.28 ^b	12.79 ^d	1.92 ^c	-49.29 ^a	-25.45 ^a	-2.56 ^a	55.53 ^a
30	32.86 ^d	16.96 ^c	1.71 ^{ab}	17.05 ^c	2.56 ^{bc}	-41.08 ^b	-21.21 ^b	-2.13 ^b	46.28 ^b
45	43.82 ^a	22.62 ^a	2.28 ^a	22.73 ^a	3.41 ^a	-30.12 ^c	-15.55 ^d	-1.56 ^d	33.93 ^c
60	33.78 ^d	17.44 ^c	1.75 ^{ab}	17.52 ^c	2.63 ^{abc}	-40.16 ^b	-20.73 ^b	-2.09 ^b	45.24 ^b
120	36.82 ^c	19.01 ^b	1.91 ^{ab}	19.10 ^b	2.86 ^{ab}	-37.12 ^c	-19.16 ^c	-1.93 ^{bc}	41.82 ^c
180	38.14 ^b	19.69 ^b	1.98 ^a	19.79 ^b	2.97 ^{ab}	-35.80 ^d	-18.48 ^c	-1.86 ^c	40.33 ^d

L* = lightness; a* = red-green values; b* = blue-yellow values; C* = chroma; h* = angle; ΔL , Δa , Δb = changes after heating; ΔE = total color change; different letters indicate significant differences between means at ** $p < 0.01$, *** $p < 0.001$

Table 4. The changes of colour parameters in blueberry extract at 70°C/180 min

Time (min)	L*	a*	b*	C*	h*	ΔL	Δa	Δb	ΔE
	***	***	***	***	***	***	***	***	***
15	20.61 ^e	8.32 ^d	4.74 ^e	9.58 ^d	9.89 ^d	-41.22 ^a	-16.64 ^a	-9.48 ^a	41.45 ^a
30	27.48 ^d	11.09 ^c	6.33 ^d	12.77 ^c	13.19 ^c	-34.35 ^b	-13.87 ^b	-7.90 ^b	37.88 ^b
45	36.64 ^a	14.79 ^a	8.43 ^a	17.03 ^a	17.58 ^a	-25.19 ^c	-10.17 ^d	-5.79 ^c	27.78 ^f
60	28.24 ^d	11.40 ^c	6.50 ^{cd}	13.12 ^c	13.55 ^c	-33.59 ^b	-13.56 ^b	-7.72 ^{bc}	37.04 ^e
120	30.79 ^c	12.43 ^b	7.08 ^{bc}	14.31 ^b	14.77 ^b	-31.04 ^c	-12.53 ^c	-7.14 ^{cd}	34.23 ^d
180	31.89 ^b	12.87 ^b	7.33 ^b	14.82 ^b	15.30 ^b	-29.94 ^d	-12.09 ^c	-6.89 ^d	33.02 ^e

L* = lightness; a* = red-green values; b* = blue-yellow values; C* = chroma; h* = angle; ΔL , Δa , Δb = changes after heating; ΔE = total color change; different letters indicate significant differences between means at *** $p < 0.001$



Heat treatment of anthocyanins could result in browning as well as in colour loss depending on heat induced reactions of anthocyanins which include deglycosylation, opening of the pyrylium ring, formation of chalcone and generation of C6–C3–C6 structure fragments. During thermal processing, anthocyanins can also polymerize which could improved colour stability. In this work, all extracts after heating showed noticeable colour changes. The maximum total colour change was determined for blackberry followed by blueberry and mulberry.

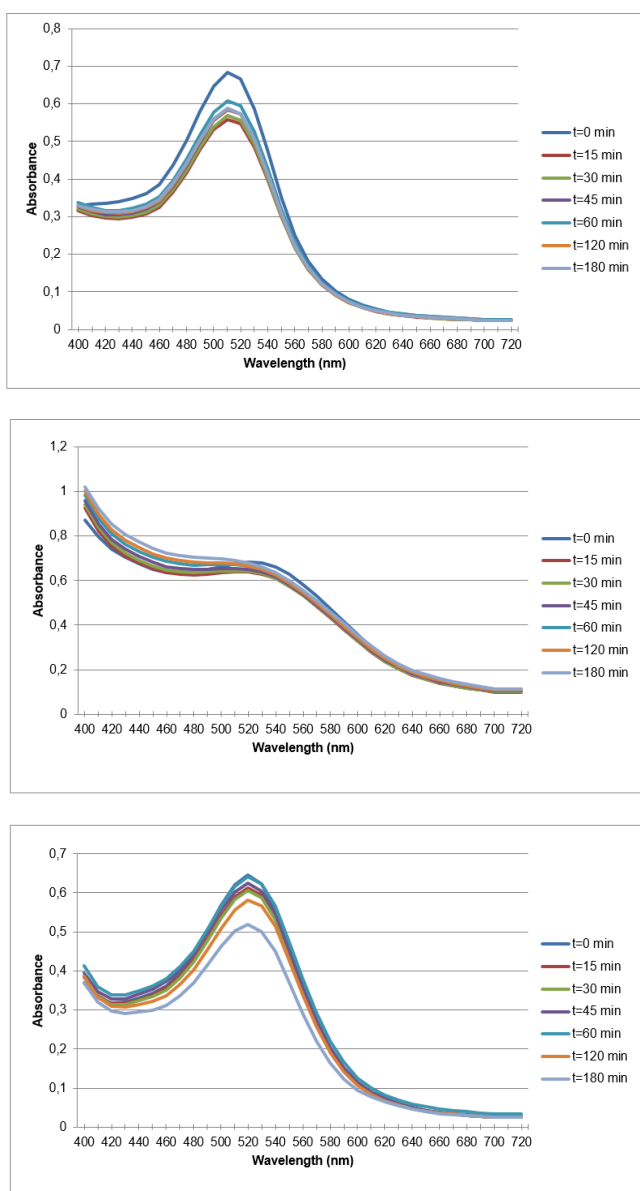


Figure 3. Absorption spectra of blackberry (a), mulberry (b) and blueberry extract (c) at 70°C

Generally, the colour degradation is a function of temperature and processing time. Colour degradation increased with prolonged heating resulting in modifications in the absorption spectra (400–700 nm) of the extracts (Fig. 3 a, b, c). From the presented absorption spectra, it can be concluded that the mulberry extract was the most thermostable. Colour degradation of blackberry extracts occurred after 15 minutes at 70°C and thereafter remains relatively stable. After 3 hours of heating at 70°C blackberry extract has retained 86.6% of the absorbance

at 520 nm, blueberry extract 76.1%, and mulberry extract even 97.51% (Fig. 4).

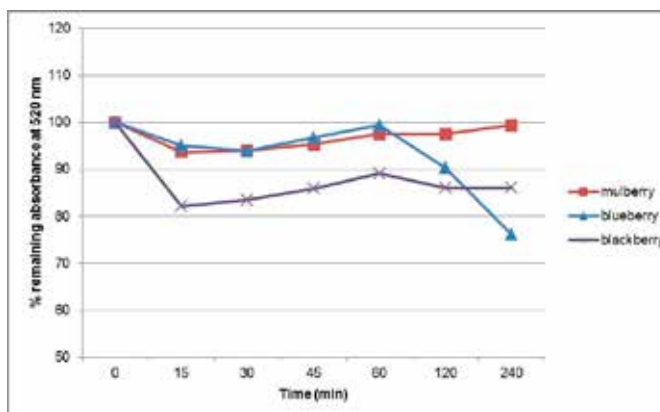


Figure 4. Remaining absorbance at 520 nm as a function of the heating time

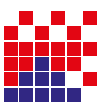
Also, it is evident that the colour of blueberry extract in the first hour of heating at 70°C was stable but afterwards there is a fast change and fall in absorbance. The colour change as well as degradation of colour pigments are expected due to the fact that increased temperature in combination with prolonged processing time have strong effect on thermo labile components (Fernandez-Lopez et al., 2013). Obtained different colour stability of blackberry, mulberry and blueberry extracts could be explained by the different chemical structure of present anthocyanins, including the aglycone type as well as bonded sugar type.

Conclusions

Based on the results of this study it could be concluded that the examined berry fruits (mulberry, blackberry and blueberry) are characterized as rich source of natural antioxidants, phenolic compounds, especially anthocyanins. The highest amount of anthocyanins was found in blackberry extracts. Results of analysed antioxidant activity by the ABTS and DPPH method were correlated to the amounts of total phenolics. These berry fruits are abundant in anthocyanin pigments and may find wider application in the food industry as natural colourants. Obtained results showed that the type of extract and the duration of heating had influence on colour stability. Mulberry extract exhibited the highest thermostability at 70°C during 3 hours.

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