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Evaluation of Quercetin Content, Colour and Selected Physico-Chemical Quality Parameters of Croatian Blackberry Wines

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Key words: blackberry wine, conventional or organically grown, quercetin, colour, physico-chemical parameters

Blackberry wine, the most widespread Croatian fruit wine, is produced by yeast fermentation of raw blackberry juice or must. Even though the production and intake of blackberry wine in Croatia has been increasing for years, the available data on the overall quality and blackberry wine composition is still scarce. The objective of this work was to evaluate the quercetin content, colour and selected physico-chemical quality parameters of 15 Croatian blackberry wine samples, divided into two groups denominated as CWB and OWB regarding the blackberries' cultivation type (conventional and organic). The quercetin content ranged from 0.81 to 21.67 mg/L (average: 6.16 mg/L, median 3.96 mg/L) and was consistent with some published studies. Compared to CBW group, quercetin content of OBW samples seemed to be more balanced and slightly higher (OBW median: 3.99 mg/L, CWB median: 1.80 mg/L). The presence of cyanidin and pelargonidin (as aglycons) was detected in very low concentrations in four and seven samples, respectively. Determination of the colour revealed that the most important component contributing to colour intensity was yellow (51 %), followed by red (40 %). The chemical quality parameters were in accordance with the available published results on blackberry and grape wines and were as follows: relative density 1.0017–1.0660 g/mL, concentration of reducing sugars 13.5–177.6 g/L, alcoholic strength 9.37–14.78% vol, pH 3.11–3.56, total acidity 6.7–18.1 g/L, ash 1.59–4.11 g/L, alkalinity of ash 1.54–3.79 g/L, total nitrogen 65.50–361.15 mg/L, and total phosphorus 32.03–118.53 mg/L. No significant overall differences were noticed between the conventional and organic group of samples.

INTRODUCTION

Fruit wine is defined as wine fermented from juice or skin of fresh fruits other than grapes. The type, production and consumption of fruit wines differ depending on the geographical area and fruit cultivars typically grown in that specific area. Fruit wines typical for Croatia are those produced from berries (blackberry, raspberry, blueberry, elderberry), as well as from cherries and apples. Blackberry wine (BW), the most widespread Croatian fruit wine, is produced by yeast fermentation of raw blackberry juice or must. The quality of blackberry wine depends on numerous factors such as plant variety/cultivar, soil and climate, cultivation type, winemaking practices, *etc*. The trends in the food industry and the recommendations of nutritionists are tending towards the development of new functional fruit-based products, which are beneficial to human health and contribute to the prevention of degenerative processes caused by oxidative stress [Lopez *et al*., 2007]. Fruits and their products such as fruit wines contain many dietary phytonutrients such as flavonoids, phenolic acids, carotenoids and vitamins, exhibiting strong antioxidant potential [Bowen-Forbes *et al*., 2010]. Fruit wine colour, taste and astringency are the sensory properties predominantly influenced by the phenolic compounds presence [Yildirim, 2006]. Berries are a rich source of flavonoids, out of which quercetin is the major dietary representative of the flavonol subclass of flavonoids [Hollman *et al*., 1997; Nijveldt *et al*., 2001]. In recent years, the production and intake of blackberry wine, both organic and conventional, has increased substantially in Croatia. However, the vast majority of producers are small-scale family businesses that have difficulties providing quantities needed for the growing market. Therefore, blackberry wines are still commercially underrepresented. Furthermore, there has been little systematic analysis of the overall quality (physico-chemical quality parameters and sensorial characteristics) or production technology of blackberry wines in Croatia or elsewhere [Yildirim, 2006; Amidžić Klarić *et al*., 2011a,b, 2015; Gao *et al*., 2012; Johnson & Gonzalez de Mejia, 2012; Arozarena, 2012; Ortiz, 2013].

The aim of this study was to investigate the quercetin content, colour and selected physical-chemical quality parameters of Croatian blackberry wine samples, made from blackberries from both conventional and organic cultivation. Furthermore, two major anthocyanidins: cyanidin and pelargonidin as aglycons were also determined.

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MATERIALS AND METHODS

Blackberry wine samples

The quercetin, cyanidin and pelargonidin contents, colour and selected physico-chemical characteristics of blackberry wines were evaluated in 15 samples of young blackberry wines (bottled and analysed within a year of their vintage) originating from small Croatian producers. The samples were delivered to the laboratory as correctly sealed, triplicate bottles. They were stored at +4°C and opened upon the analysis. The samples were divided into two groups, conventional and organic, according to the blackberries' cultivation method. The conventional group consisted of seven samples (CBW 1 – CBW 7) while eight samples (OBW 8 – OBW 15) were produced from organically grown blackberries that were cultivated according to the EU organic cultivation legislation [Commission Implementing Regulation (EU) No 203/2012; Commission Regulation (EC) No 889/2008].

Chemicals

Standard of quercetin dehydrate was supplied from Riedel – de Haën (Seelze, Germany), cyanidin chloride, pelargonidin chloride and glycine standards were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany), while a standard phosphorus solution (concentration 1000±0.002 mg/L) was provided from AccuTrace™ (New Haven, CT, USA). Methanol was purchased from Carlo Erba Reagent (Milan, Italy), acetonitrile was obtained from Baker (J.T. Baker, Milan, Italy) and glacial acetic acid was supplied by Panreac (Barcelona, Spain). Kjeltabs Cu/3.5 (3.5 g K_2SO_4 and 0.4 g $CuSO_4 \times 5H_2O$), which was used as a catalyst, was obtained from Foss Tecator (Höganäs, Sweden). Ammonium molybdate tetrahydrate was provided from Himedia (Mumbai, India) while ammonium heptamolybdate tetrahydrate and ammonium mono vanadate were from Analytics Ltd. (Ke Kličovu, Czech Republic). Lead (II) acetate trihydrate, calcium carbonate, copper (II) sulphate pentahydrate, potassium iodide, boric acid and other basic chemicals were purchased from Kemika (Zagreb, Croatia). All reagents used in this work were of analytical reagent grade or better while all solvents were HPLC grade. Double deionised water (DDW) from a WaterPro water system (Labconco Corporation, Kansas City, MO, USA) with a resistivity of 18.2 MΩ cm (25°C) was used in all experiments.

Quercetin and anthocyanidins determination

Quercetin and anthocyanidins (cyanidin and pelargonidin) were determined by high-performance liquid chromatography (HPLC). The chromatographic analyses were performed using a Dionex chromatographic system (Sunnyvale, CA, USA) consisting of P680 pumping system, ASI 100 automatic sample injector, TCC-100 oven for columns, UVD170S detector and Chromeleon 6.8 software. Fifteen samples of blackberry wine were injected (20 μL) into the column directly without extraction and hydrolysis (anthocyanidins) and after hydrolysis (quercetin). All samples were separated on a Lichrospher 100-RP18 column (250 × 4 mm, 5 μm; Agilent Technologies, Santa Clara, CA, USA) with a suitable guard column (LiChrospher 100-RP18, 4 × 4 mm, 5 μm; Agilent Technologies, Santa Clara, CA, USA). The chromatographic conditions were determined according to a modified procedure by Gambelli & Santaroni [2004]. The solvents used for gradient elution were (A) acetic acid: water (5: 95) and (B) acetonitrile. The concentration of acetonitrile increased from 0% to80 % during 30 min and during the next 3 min decreased to 0% (total run time, 33 min). Elution was carried out at a flow rate of 1 mL/min and the column was thermostatically controlled to maintain the temperature of 30°C. During each run, the absorbance was recorded at 370 nm (quercetin) and 520 nm (cyanidin and pelargonidin). Pure standards of quercetin and anthocyanidins in methanol (10 mg/L) were used to construct the standard curve and to determine retention times. Quantitative analysis was performed in triplicate using external calibration curves.

Hydrolysis conditions

Quercetin glucosides were completely hydrolysed to aglycons according to slightly modified methods described by Vuorinen *et al*. [2000] and Gambelli & Santaroni [2004]. Briefly, 2–5 mL of each investigated blackberry wine was diluted with 50% methanol (v/v) containing hydrochloric acid (0.5 mol/L) using the appropriate dilution. The mixture was incubated in a water bath at 80°C for 2 h. The samples were filtered through a 0.2 μm filter (Sartorius, Göttingen, Germany) prior to the injection of 20 μL of samples to HPLC.

Colour determination

Spectral readings over the visible spectrum, 380–780 nm, and absorbance measurements at 420, 520 and 620 nm were conducted on a UV-visible Lambda 25 spectrophotometer using quartz cells with a 1-mm path length (Perkin-Elmer, Waltham, MA, USA). This spectrophotometer has the necessary UV WinLab 6.0.2.0723 software (Perkin-Elmer, Waltham, MA, USA) to calculate the CIELAB parameters [CIE, 1986] directly. Before the chromatic analysis, all samples were filtered through Minisart RC4, 0.45-μm filters (Sartorius, Germany), which did not retain any of the analytes. The colour intensity (I), the proportion of yellow (dA_{420} (%)), red (dA_{520} (%)) and blue (dA_{620} (%)) colour and the proportion of red colour produced by the flavylium cations of free and bound anthocyanins (dA (%)) were calculated using the following equations (1–6) by Glories [1984] and Sudraud [1958].

$$I = \sum (A_{420} + A_{520} + A_{620}) \quad (1)$$

$$T = \frac{A_{420}}{A_{520}} \quad (2)$$

$$dA_{420}(\%) = \frac{A_{420}}{I} \times 100 \quad (3)$$

$$dA_{520}(\%) = \frac{A_{520}}{I} \times 100 \quad (4)$$

$$dA_{620}(\%) = \frac{A_{620}}{I} \times 100 \quad (5)$$

$$dA(\%) = \left[A_{520} - \left(\frac{A_{420} + A_{620}}{2} \right) \right] \times \left(\frac{1}{A_{520}} \right) \times 100 \quad (6)$$

Physico-chemical quality parameters determination

Density

Density was determined according to the reference OIV method, using a pycnometer set at 20°C [O.I.V., 2008].

Ash and alkalinity of ash

Measurements of ash and alkalinity of ash were carried out using the official OIV methods [O.I.V., 2008]. The blackberry wine samples were ignited at temperatures ranging from 500°C and 550°C until complete oxidation of organic material has been achieved. After that, the ash from a 20 mL sample was dissolved in 10 mL of 0.05 mol/L sulphuric acid solution, and the excess was determined by titration using 0.1 mol/L sodium hydroxide and 0.1% methyl orange as an indicator.

Reducing sugars

The content of total reducing sugars was determined by iodine titration of excess copper using the Luff-Schoorl method [O.I.V., 2008]. Before titration, the samples were clarified using a neutral lead acetate solution. The sugar content of the blackberry wine samples was expressed as grams of invert sugar per litre, taking into account the dilution made during clarification and the test sample volume.

pH determination

The digital pH meter MP 225 with a combination pH electrode INLAB 413 (Mettler Toledo, Switzerland) was used to determine the pH values of blackberry wine samples [O.I.V., 2008].

Total acidity

The total acidity was determined by potentiometric titration with standardised sodium hydroxide [O.I.V., 2008], and the results were expressed as grams of tartaric acid per liter.

Alcoholic strength by volume

After distillation, the alcohol content was determined with a pycnometer according to the OIV method [O.I.V., 2008].

Determination of total nitrogen

The total nitrogen content of the blackberry wines was determined according to Kjeldahl method [O.I.V., 2008]. A semi-automatic analysis unit Kjeltec TM 2300 equipped with a Digestor 2006, Scrubber 2001 and Controller 2000 (Foss Tecator, Sweden) was used for the determination of total nitrogen in the samples.

Determination of total phosphorus

The total phosphorus concentration was estimated using a colorimetric assay based on the procedure described by OIV [O.I.V., 2008] and Zoecklein [1995]. Prior to spectrometric analysis, each sample was wet-ashed using sulphuric and nitric acids. After that, the sample solution was diluted with DDW, and a 10-mL sample aliquot was added to 10 mL of vanadomolybdate reagent. The mixture was vortexed for 15 s and then left to stand at room temperature for 10 min. The absorbance of the resulting solution was measured at 430 nm using a spectrophotometer (model Lambda 25 UV-VIS; Perkin-Elmer, USA). The results were determined from a standard calibration curve and expressed as mg/L.

Statistical analysis

All determinations were conducted in triplicate, and the obtained data presented as the means ± standard deviations of parallel investigations. First, all data were tested for normal distribution using the Kolmogorove-Smirnov test. The variables with a normal distribution are described by the arithmetic mean and standard deviation, and those not showing a normal distribution are presented by the median and interquartile range. The univariate characterisation of blackberry wines by the blackberries' cultivation method was carried out using a t-test for variables with a normal distribution and non-parametric statistics (the Mann-Whitney U test) for others. The Pearson product-moment correlation coefficient and Spearman rank correlation coefficient were determined to examine potential relationships between the concentrations of different compounds. $P<0.05$ was considered statistically significant and $p<0.01$ very significant. The statistical package STATISTICA ver. 7.1 from StatSoft® (Tulsa, OK, USA) was used for analyses of all data.

RESULTS AND DISCUSSION

The results of quercetin and anthocyanidins content and physico-chemical quality parameters determination of blackberry wine samples are summarised in Table 1. No significant differences were found between the triplicates.

Quercetin and anthocyanidins determination

A satisfactory separation was achieved using the above-described method. The representative chromatograms are presented in Figures 1 and 2. The proposed method for the determination of quercetin and anthocyanidins (cyanidin and pelargonidin) in blackberry wine samples was validated according to the recommendations of the International Conference on Harmonization (ICH) guidelines [International Conference on Harmonization, 2005]. The performance characteristics of the method were evaluated on the basis of linearity, detection limit (LOD), quantification limit (LOQ), precision and accuracy. The linearity for these compounds was examined by analysing the solutions within the concentration range from 0.25 mg/L and 10 mg/L. The correlation coefficient of the linear regression of the standard curves was 0.9999 for all investigated compounds. Detection and quantification limits were determined using progressively lower concentrations for a signal-to-noise ratio of 3:1 and 10:1, respectively (LOD=0.05 mg/L and LOQ=0.15 mg/L for quercetin; LOD=0.06 mg/L and LOQ=0.22 mg/L for cyanidin; and LOD=0.06 mg/L and LOQ=0.19 mg/L for pelargonidin). The accuracy of the method was investigated by multiple injections (n=3) of three different standard solutions. The recovery rate for quercetin was 98.8±0.4%, for cyanidin was 96.1±0.7%, and for pelargonidin was 97.4±0.9%. The repeatability of the method was tested by repeated injections of the standard solutions mixture (n=3) over three consecutive days. The relative standard deviations (RSD) were

TABLE 1. Quercetin and anthocyanidins content and physico-chemical quality parameters of blackberry wine samples.

Sample	Quercetin (mg/L)	Cyanidin (mg/L)	Pelargonidin (mg/L)	Relative density (g/mL)	Reducing sugar (g/L)	Alcoholic strength by volume (% vol)	pH	Total acidity (g/L)	Ash (g/L)	Alkalinity of ash (g/L)	Total N (mg/L)	Total P (mg/L)
CBW 1	1.01±0.01	<LOD	<LOD	1.0070±0.0001	30.0±1.2	14.78±0.02	3.33±0.01	6.7±0.1	1.58±0.03	1.54 ±0.02	65.50±2.89	32.03±0.09
CBW 2	0.81±0.01	<LOD	<LOD	1.0558±0.0005	144.3±0.8	9.37±0.30	3.43±0.01	16.3±0.1	3.13±0.00	3.03±0.04	307.60±3.86	88.40±0.14
CBW 3	1.80±0.02	<LOD	<LOD	1.0660±0.0005	176.9±1.2	10.78±0.21	3.45±0.02	10.9±0.0	2.30±0.14	2.87±0.07	134.49±3.57	118.53±0.16
CBW 4	10.35±0.01	<LOD	1.46±0.02	1.0218±0.0001	50.6±1.4	12.86±0.02	3.20±0.01	15.9±0.0	4.04±0.10	3.80±0.03	127.28±0.39	81.98±0.16
CBW 5	21.67±0.02	3.22±0.02	1.22±0.04	1.0286±0.0001	101.8±1.4	13.64±0.01	3.11±0.01	18.1±0.0	2.85±0.07	2.84±0.01	168.83±2.25	59.97±0.08
CBW 6	15.93±0.02	<LOD	0.63±0.01	1.0312±0.0002	84.9±0.6	12.97±0.02	3.31±0.01	14.8±0.0	3.21±0.06	3.19±0.02	261.43±6.96	80.52±0.17
CBW 7	1.25±0.02	0.92±0.01	<LOD	1.0143±0.0001	29.4±2.2	14.63±0.01	3.13±0.01	14.8±0.0	2.26±0.04	2.21±0.05	77.45±3.51	69.14±0.16
OBW 8	3.96±0.01	<LOD	<LOD	1.0131±0.0004	39.0±1.2	14.12±0.40	3.46±0.01	11.3±0.0	3.05±0.05	3.16±0.05	164.84±1.09	37.72±0.13
OBW 9	3.36±0.03	<LOD	<LOD	1.0648±0.0006	177.6±0.1	12.06±0.01	3.25±0.02	12.2±0.0	3.34±0.01	3.31±0.02	261.11±3.65	108.82±0.22
OBW 10	4.03±0.01	<LOD	<LOD	1.0441±0.0001	124.1±0.7	13.50±0.01	3.47±0.01	9.2±0.0	3.08±0.04	3.05±0.03	341.01±3.33	42.63±0.14
OBW 11	8.05±0.09	1.48±0.02	1.42±0.01	1.0312±0.0001	87.6±2.4	13.66±0.01	3.24±0.01	11.7±0.0	2.64±0.05	2.65±0.04	142.35±1.98	105.96±0.17
OBW 12	1.53±0.02	<LOD	<LOD	1.0127±0.0002	50.8±0.6	12.32±0.02	3.20±0.02	11.6±0.0	4.10±0.07	3.79±0.07	274.64±7.70	63.39±0.21
OBW 13	7.55±0.02	0.84±0.02	1.05±0.04	1.0171±0.0003	65.5±1.5	14.38±0.06	3.56±0.00	7.9±0.0	3.10±0.09	3.05±0.02	123.56±5.26	103.59±0.21
OBW 14	2.23±0.04	<LOD	0.89±0.01	1.0050±0.0001	28.5±1.2	12.05±0.04	3.38±0.01	9.3±0.0	3.27±0.07	3.19±0.02	97.15±2.81	45.97±0.15
OBW 15	8.92±0.03	<LOD	0.99±0.01	1.0017±0.0001	13.5±1.3	13.34±0.01	3.46±0.02	13.2±0.0	3.69±0.08	3.64±0.02	361.15±3.46	103.08±0.19

Legend: values are presented as means of three parallel investigations ± standard deviation (data: mean±SD, n = 3). CBW – conventional blackberry wine; OBW – organic blackberry wine; N – nitrogen; P – phosphorus; LOD – limit of detection. No significant differences were found between the triplicates for all determinations.

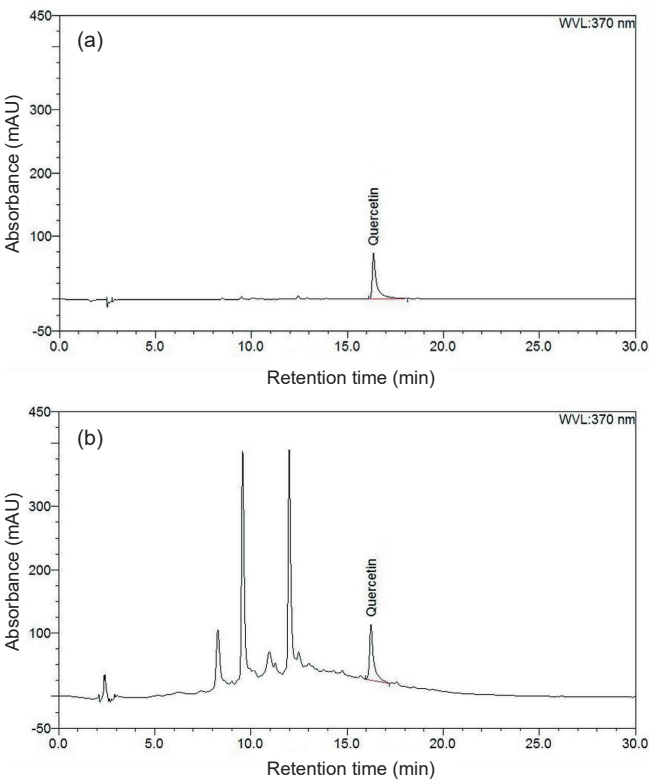

FIGURE 1. (a) HPLC chromatogram of a standard solution (standard no. 4: quercetin 5 mg/L) recorded at 370 nm. (b) Chromatogram of blackberry wine (CBW 6) after hydrolysis monitored at 370 nm.

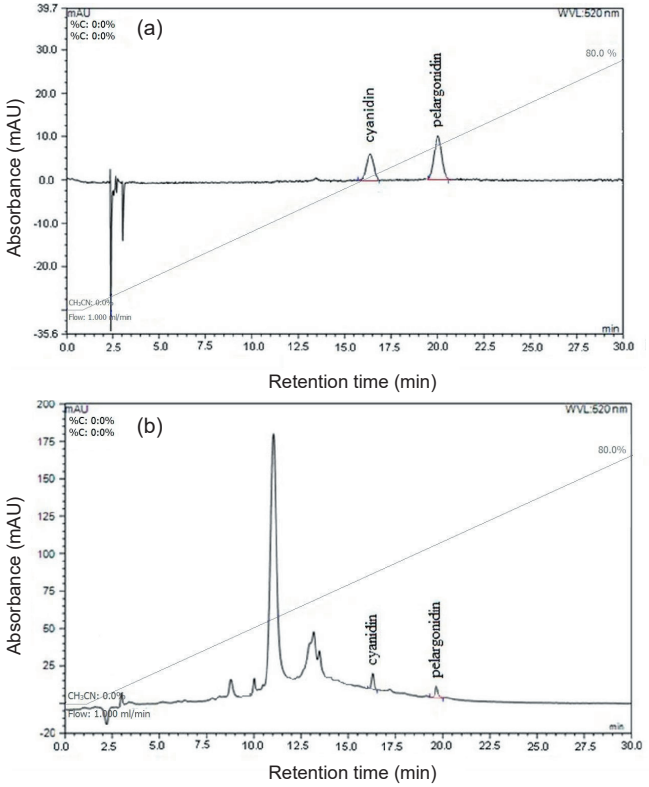

FIGURE 2. (a) HPLC chromatogram of a standard solution (standard no. 3: 3 mg/L) recorded at 520 nm. (b) Chromatogram of blackberry wine (CBW 3) monitored at 520 nm.

in the range from 0.8% to 2.76% for intra-day repeatability and from 0.96% to 3.39% for inter-day repeatability. These results clearly show that the chromatographic method was suitable for the determination of quercetin and anthocyanidins (cyanidin and pelargonidin) in blackberry wine samples. Since the determinations were performed after acid hydrolysis, the total quercetin concentration (aglycone and glycosides) was determined, as opposed to usually determined quercetin glycosides. The quercetin content ranged from 0.81 to 21.67 mg/L (average: 6.16 mg/L, median 3.96 mg/L). Three samples (CBW 4, 5 and 6) had concentrations of quercetin higher than 10 mg/L. Even though the quercetin content varied over a wide range, the results are in agreement with the published data for berry and grape wines [McDonald *et al*., 1998; Vuorinen *et al*., 2000; Tsanova-Savova & Ribarova, 2002]. Compared to CBW group, quercetin content of OBW samples seemed to be more balanced and slightly higher (OBW median: 3.99 mg/L, CWB median: 1.80 mg/L).

The presence of cyanidin, as aglycon, was detected in four blackberry wine samples (CBW 5, CBW 7, OBW 11 and OBW 13), and the values obtained were within the range of 0.84 mg/L to 3.22 mg/L (RSD to 2.2%). The concentration was below the LOD (0.11 mg/L) in all the remaining samples. Furthermore, the presence of pelargonidin was determined in three conventional (CBW 4, CBW 5 and CBW 6) and four organic (OBW 11, OBW 13, OBW 14 and OBW 15) samples and was in the range 0.63–1.46 mg/L (RSD to 3.39 %). In all the remaining samples the concentration was below the LOD (0.06 mg/L). The low concentrations of cyanidin and pelargonidin could be attributed to the fact that in nature anthocyanidins are generally present in the form of glycosides – anthocyanins [Arozarena, 2012; Ortiz, 2013; Castañeda-Ovando *et al.*, 2009].

Colour determination

Colour is one of the main parameters taken into account when evaluating the wine quality. It is not always easy to objectively define and evaluate wine colour. Colorimetric assays were carried out on two groups of investigated blackberry wines according to Glories (standard) and CIELAB procedures and the summarised results are presented in Table 2. The CIE method provides a more precise definition than the Glories and OIV methods because the CIE method uses measurements over the complete visible spectrum (380––780 nm), similar to what is perceived by the human eye. On the other hand, the Glories method is a simple alternative to colour components measurement that enables simple measurement of colour components. Colour intensity of investigated blackberry wines ranged from 1.8 to 13.19 and was in accordance or even slightly higher than the results reported in other studies [Arozarena *et al*., 2012; Ortiz *et al*., 2013]. Yellow and red were the components that on average accounted for more than 90% of the colour intensity for both CBW and OBW sample groups. The contribution of blue to the overall colour intensity was on average 6.13 and 6.35 for CBW and OBW samples, respectively. This is also similar to the results reported by Ortiz *et al*. [2013] and Arozarena *et al*. [2012]. In this study, however, red was not the most important component contributing to colour intensity, but yellow. When compared to results of the above-mentioned studies where

TABLE 2. Colour parameters statistical summary of blackberry wine samples.

Parameter	Conventional samples group				Organic samples group			
	Mean±SD	Median	Range of quantified values	Interquartile	Mean±SD	Median	Range of quantified values	Interquartile
A_{420}	3.19±1.47	3.49	1.01–5.15	1.89–4.62	3.41±0.77	3.48	2.16–4.35	2.91–4.01
A_{520}	3.09±2.27	2.31	0.79–6.47	1.08–6.05	2.78±0.94	2.47	1.58–4.15	2.20–3.57
A_{620}	0.606±0.663	0.294	-0.010–1.570	0.006–1.395	0.443±0.247	0.374	0.158–0.800	0.236–0.683
Color Intesity (I)	6.88±4.31	6.11	1.80–13.19	2.98–12.07	6.66±1.90	6.46	3.89–9.42	5.53–8.00
Wine Hue	1.226±0.366	1.276	0.763–1.740	0.796–1.571	1.273±0.193	1.264	1.048–1.530	1.091–1.448
X	23.3±21.9	21.7	1.7–55.1	2.1–48.3	17.7±8.4	17.8	6.6–30.8	10.2–24.0
Y	14.9±15.9	11.5	0.7–40.7	0.9–32.6	9.1±5.1	8.6	3.5–18.2	4.5–12.5
Z	2.274±4.555	0.110	0.000–12.320	0.000–2.943	0.280±0.425	0.095	0.100–1.237	0.045–0.357
x	0.633±0.069	0.650	0.510–0.702	0.576–0.698	0.661±0.028	0.659	0.613–0.694	0.645–0.687
y	0.342±0.034	0.345	0.298–0.389	0.302–0.376	0.331±0.020	0.336	0.306–0.362	0.312–0.344
L*	36.5±25.5	40.5	6.5–70.0	8.2–63.9	34.6±9.9	35.2	22.0–49.7	25.2–42.1
a*	49.2±13.4	46.7	34.4–69.3	36.0–62.1	60.5±8.2	62.5	41.4–67.2	60.0–65.2
b*	46.9±27.3	51.0	11.2–77.4	14.1–71.1	55.6±13.2	59.4	35.7–69.8	43.4–66.9
Chroma (Cab*)	69.2±27.0	69.2	36.2–99.3	38.7–95.0	82.4±13.9	88.2	54.7–95.1	74.1–92.6
S	2.827±1.687	2.283	0.990–5.563	1.487–4.737	2.465±0.371	2.448	1.830–2.933	2.253–2.777
Q	62.8±22.9	66.4	36.9–93.0	37.4–87.5	61.1±8.9	61.7	49.8–74.7	52.7–67.9
Hue angle (hab*)	39.4±14.1	45.7	18.0–54.6	21.3–47.7	42.1±4.8	42.6	35.9–48.4	38.1–45.8
dA_{420} (%)	50.9±9.5	51.2	38.3–63.4	39.1–58.2	52.1±4.6	51.6	46.2–57.9	48.2–56.4
dA_{520} (%)	42.9±5.8	44.2	36.4–50.2	37.0–49.0	41.3±3.0	41.4	37.6–45.2	38.3–44.0
dA_{620} (%)	6.13±5.58	4.81	-0.57–11.91	0.19–11.89	6.35±2.74	5.64	4.07–12.35	4.27–7.34
dA%	31.7±15.9	36.8	12.7–50.4	15.0–48.0	28.6±9.3	29.0	17.0–39.4	19.5–37.8

Legend: values are presented as means of three parallel investigations ± standard deviation (data: mean±SD, n = 3). $A_{420,}$ $A_{520,}$ A_{620} – A were measured at 420 nm; 520 nm and 620 nm; dA% – % of monomeric anthocyanins. No significant differences were found between the triplicates for all determinations.

red was almost twice higher than yellow, in this study the difference between red and yellow was not that evident. On average, yellow accounted for 51% of colour intensity, while red accounted for around 40% for both sample groups.

As wine colour is strongly dependent on the pH [Zocklein *et al*., 1995; Vivar-Quintana *et al*., 2002; Wrolstad *et al*., 2005], it is interesting to note that statistical analyses have demonstrated significant correlations between pH value and hue (r=0.7107; p=0.003), pH value and proportion of red dA_{520} (r=–0.7662; p=0.001), and pH and the proportion of red colour produced by the flavylium cations of free and bound anthocyanins dA% (r=–0.7334; p=0.002), which is in agreement with the previous statement. Knowing the fact that different wines have different pH values, the utility of these measurements is very limited for different wine comparison.

Physico-chemical quality parameters

As seen in Table 1, the relative density of blackberry wine samples ranged from 1.0017 g/mL (OBW 15) to 1.0660 g/mL (CBW 3) and the mean value of all investigated samples was 1.0276 g/mL. Furthermore, it has been observed that the mean value of this physical characteristic was similar for both groups – conventional and organic. Density is an indicator of wine quality that depends on several factors: the ethanol extract, the sugar content and the glycerol content [Zoecklein *et al*., 1995; Dı az *et al*., 2003]. Statistical analysis demonstrated a significant correlation (r=0.9849, p<0.001) between the relative density of blackberry wine and the content of reducing sugars. The concentration of reducing sugars (Table 1) found in the blackberry wines varied over a wide range from 13.5 g/L (OBW 15) to 177.6 g/L (OBW 9), and the median of all investigated BWs was 65.5 g/L. However, no statistically significant difference in the concentration of reducing sugars was noted between the two studied groups of blackberry wines. Reducing sugars, glucose and fructose are widely present in foods, especially fruits. Blackberries usually contain 4.9 g sugars per 100 g [Huerta *et al*., 1998; USDA], and during wine fermentation, both monosaccharides are co-ferment-

ed by yeasts, producing diverse compounds such as carbon dioxide, ethanol and glycerol. Furthermore, sugar (mostly saccharose) is added during different stages of the blackberry wine production process, so the wide range of reducing sugar concentrations found in the investigated samples is probably the result of the applied technological procedure. Ethanol is the main product of alcoholic fermentation, and its content in grape wines usually varies from 7 to 24% [Jacobson, 2006], while methanol and other alcohols are significantly underrepresented. Ethanol is important for both flavour and stability of wine. Johnson & Gonzalez de Mejia [2012] reported the ethanol percentage range from 9.5 to 12.4% (10.7% average) in investigated Illinois blackberry wines. The alcoholic strength of the tested Croatian blackberry wines ranged from 9.37% (CBW 2) to 14.78% (CBW 1) by volume (Table 1), and no statistically significant difference in alcoholic strength between the two groups of the analysed samples was observed. Significant correlation ($r=0.9394$; $p<0.0001$) between alcoholic strength and previously published ethanol content data [Amidžić Klarić *et al*., 2015] was observed. The small difference between the amount of ethanol (14.99% vol) and alcoholic strength (14.78% vol) in the sample CBW 1 could be explained by less precision of pycnometer method after distillation than GC-FID method, as well as by the fact that homologues of ethanol, together with the ethanol and esters of ethanol homologues are included in the alcoholic strength since they occur in the distillate [O.I.V., 2008]. However, all the remaining samples showed the agreement between the results obtained by the two methods, which is evident when mean and median of the two studied groups of blackberry wines are compared. In addition to reducing sugars and alcohols, acids are also an important component of fruit, must and wine. The content of organic and inorganic acids in fruit depends on the fruit species, climate and geomorphological character and varies over a wide range. The acidity of blackberries changes during growth and ripening and affects the acidity of blackberry juice and must. Consequently, the acidity of must can affect wine because some acids from must transfer to wine and participate in different physico-chemical and biochemical processes, such as wine flavour formation [Zoecklein *et al*., 1995; Huerta *et al*., 1998]. The first indicator of wine acidity is the pH value (Table 1), which ranged from 3.11 (CBW 5) to 3.56 (OBW 13) in the investigated blackberry wines. The obtained results are consistent with our previous research results [Amidžić Klarić *et al*., 2011a], as well as with the results reported by other authors [Johnson & Gonzalez Mejia, 2012; Gao *et al*., 2012]. The conventional wine group samples were slightly more acidic (mean value: 3.28) than the organic wine samples (mean value: 3.38), which was confirmed by the results of total acidity determination by titration.

As the measure of the amount of acidity in wine, total acidity is an important characteristic influencing the wine taste and the overall quality. In grape wine testing, a high level of acidity refers to excessively tart, sour and sharp wine attributes, while a low total acidity results in a flat-tasting wine that is more susceptible to infection and spoilage by microorganisms [Zoecklein *et al*., 1995; Huerta *et al*., 1998]. The total acidity of the tested samples (Table 1) ranged from 6.7 (CBW 1) to 18.1 (CBW 5) grams of tartaric acid per litre, where the mean value of all analysed BWs was 12.3 g/L, with a median of 11.7 g/L. Conventional group samples had a higher total acid content (median: 14.8 g/L) than the organic group (median: 11.5 g/L). This is in agreement with other studies that reported the total acid content of blackberry wines from 3.3 to 8.1 g/L [Johnson & Gonzalez Mejia, 2012; Gao *et al*., 2012].

Ash, the inorganic residue remaining after wine burning, was determined gravimetrically in all analysed samples. The lowest ash content was determined in the conventional sample CBW 1 (1.58 g/L), while the highest content was determined in the organic sample OBW 12 (4.10 g/L) (Table 1). The average ash content of conventional group samples was 2.77 g/L while the organic group samples had a slightly higher average content (3.28 g/L). Since there is no available data on blackberry wine ash content, the results obtained in this study were compared to grape wines. Most wine-producing countries prescribe a minimum admissible amount of ash in white and red wines, *e.g.* 1.2 g/L and 1.6 g/L for white and red wines, respectively [Dikanović-Lučan *et al*., 1993]. According to literature data, the ash content of grape wine ranges from 1.2 g/L to 4 g/L [Dikanović-Lučan *et al*., 1993; Košmerl & Bavčar, 2003]. If the ash content of grape wine sample is less than 1.2 g/L, there is a reasonable suspicion that the wine is spurious. On the other hand, a higher quality of wine implies a higher amount of minerals and ash content.

Moreover, the alkalinity of ash found in the studied blackberry wine samples (Table 1) ranged from 1.54 grams K_2CO_3 per litre in sample CBW 1 to 3.80 g/L in sample CBW 4, with a mean value of 3.02 g/L. Although the organic group samples showed a higher ash alkalinity (mean value: 3.23 g/L) than the conventional group (mean value: 2.78 g/L), the statistical analysis did not indicate a significant difference. As mentioned above, not all food yields the same reactions during ash analysis, so the total alkalinity of the ash varies significantly depending on the food. It is important to emphasise that alkaline ash is a characteristic of wine because organic acids are converted to carbonates during sample burning, and potassium carbonate is the dominant form in wine sample ash [Ough & Amerine, 1988; Zoecklein *et al*., 1995; Díaz *et al*., 2003]. A strong statistically significant correlation was observed between the ash content and ash alkalinity ($r=0.9614$, $p<0.0001$), which is in agreement with the previous statement.

The various nitrogenous components of must and wine play important roles in fermentation, clarification and potential microbial instability [Zoecklein *et al*., 1995; Moreiraa *et al*., 2011]. Total nitrogen values were obtained from the sum of all nitrogenous compounds present in the analysed blackberry wines. The results obtained by the Kjeldahl method oscillated in a rather wide range from 65.50 mg/L (CBW 1) to 361.15 mg/L (OBW 15), and the median of all investigated BW samples was 164.84 mg/L. In general, the total nitrogen content of grape wines ranges from 70 to 900 mg of nitrogen per litre, where red wines have a significantly higher content of this element compared to white wines [Zoecklein *et al*., 1995; Moreiraa *et al*., 2011]. When compared to the above range, the results of this study clearly show that only one

sample, CBW 1, had a total nitrogen concentration less than 70 mg/L. The obtained results are consistent with our previous research results [Amidžić Klarić *et al*., 2011a].

Besides nitrogen, phosphorus is the second most frequently limiting macronutrient responsible for plant growth. Therefore, the total phosphorus content in blackberry wine primarily depends on natural sources, and the obtained results (Table 1) showed that the concentration of this macronutrient in the investigated samples varied significantly, ranging from 32.03 mg/L in CBW 1 up to 118.53 mg/L in CBW 3, with a mean of 76.12 mg/L. Because of the lack of available data on blackberry wines, the observed total phosphorus concentrations were compared to that in grape wines and the analysed blackberry wines had lower total phosphorus contents than both white (mean: 217 mg/L) and red wines (mean: 303 mg/L) [Savica *et al*., 2008].

CONCLUSIONS

The purpose of the current study was to evaluate the quercetin, cyanidin and pelargonidin content, colour and physico-chemical quality parameters of 15 Croatian blackberry wines made from fruit originated from conventional and organic cultivation. The results have shown that all the investigated physico-chemical quality parameters, as well as quercetin content, were in accordance with the published literature data on blackberry wines. Cyanidin and pelargonidin (as aglycons) were present in very low concentrations. Determination of the colour, on the other hand, revealed some differences in the proportion of components contributing to colour intensity. In this study, red was not the most important component contributing to colour intensity, but yellow. No significant overall differences were noticed between the conventional and organic group of samples. However, regarding the small sample size, caution is required when drawing conclusions on the differences between the two groups. Taken into account the continuous increase of intake and production of blackberry (fruit) wines in Croatia and elsewhere, this research will serve as a base for future studies that might explore the impact of fruit cultivation practices, as well as the overall blackberry wine production technology.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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