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Polyphenolic content, antioxidant activity and metal composition of traditional blackberry products

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ABSTRACT

In this study, commercially available traditional blackberry products, namely blackberry wines and blackberry elixirs from Coastal Croatia and Bosnia and Herzegovina, were investigated as sources of bioactive compounds, essential nutrients and metals. Samples were analysed for pH, total polyphenols and monomeric anthocyanins content, individual phenolic acids (gallic acid, chlorogenic acid, caffeic acid and *p*-coumaric acid), antioxidant activity (DPPH and ABTS), reducing power (RPA), total nitrogen and phosphorus as well as metals (K, Na, Ca, Mg, Fe, Mn, Cu, Zn, Co, Cr, Cd). The results of this study showed that the studied traditional blackberry products are rich sources of polyphenols and their consumption could increase the daily intake of dietary antioxidants in humans. In addition, both groups of traditional blackberry products could be considered safe for health and good additional sources of essential minerals such as manganese and potassium. The concentrations of the analysed undesirable toxic and potentially toxic metals in the studied blackberry products were below the maximum allowable concentrations defined by various regulations for wines and fruit wines.

Introduction

Blackberry is a popular fruit that has been shown to have physiological effects on human health and has long been recognized as a medicinal plant. Blackberry cultivation in Croatia and neighbouring countries is steadily increasing, and significant quantities of fresh fruit are directly processed into blackberry products such as jam, juice, wine or elixir.

Blackberry wine (BW), the fermented juice from fresh blackberry fruit, is a traditional product used for health promotion and disease prevention. The nutritional and biologically active compounds, as well as the toxic components in blackberry wine, come directly from the fruit or are created during fermentation and ripening. In addition to nutrients, blackberry wine contains various bioactive compounds with antioxidant activity, such as phenolic compounds, as well as small amounts of vitamins, essential minerals and trace elements (Ljevar et al., 2016; Velić et al., 2018; Velić et al., 2019). The protective effects of polyphenols are based on the binding of free radicals, which are involved in many pathological conditions and diseases, such as cardiovascular, carcinogenic, neurodegenerative and others (Meskin et al., 2002; Shi et al., 2003; Yilmaz and Toledo, 2004; Szajdek and Borowska, 2008). In addition, blackberry polyphenols are involved in the formation of the colour, flavour and aroma of this fruit wine. The most commonly used methods for the determination of individual phenolic compounds in wines are chromatographic , namely high- performance liquid chromatography (HPLC). The major advantage of these methods is their ability to separate different

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forms of phenolic compounds from a very complex matrix (Harbertson and Spayd, 2006).

Blackberry wine is usually served as a dessert wine and enjoyed in moderate amounts with meals. Traditionally, blackberry wine (often called ferrous wine) is used for convalescents, anemics and the elderly. As this popular fruit wine is mainly produced in the continental part of the country, our previous studies were conducted on commercially available Croatian blackberry wines from three different geographical regions (Amidžić Klarić et al., 2011a; Amidžić Klarić et al., 2011b) and blackberry wines produced from organically and conventionally grown blackberries (Amidžić Klarić et al., 2015; Amidžić Klarić et al., 2016; Amidžić Klarić et al., 2017; Amidžić Klarić et al., 2020).

Blackberry elixir (BE) is also available on the market as a traditional blackberry product. It is defined as a liquid medicinal preparation intended for oral use. This aqueous and/or alcoholic product contains a lower ethanol content than wine and helps improve health as a dietary supplement.

In this study, commercially available blackberry wines and blackberry elixirs from Coastal Croatia and Bosnia and Herzegovina were investigated as sources of bioactive compounds, essential nutrients and metals. Since there is a general lack of research on blackberry elixir, the aim of this work was to analyze the polyphenolic content, antioxidant activity and metal composition in commercially available blackberry elixirs produced in the mentioned areas using chromatographic and spectrometric methods.

Materials and methods

Samples

Four commercially available blackberry products were collected from the local community pharmacies and health food stores. These products were named BWCC, BECC, BWBH and BEBH according to the type of product (blackberry wine - BW or blackberry elixir - BE) and their geographical origin (Coastal Croatia - CC and Bosnia and Herzegovina - BH).

For three samples the alcohol content was declared on the bottle (BWCC 12.1%, BWBH 12.0% and BEBH 5% ethanol), and for one sample it was not declared (BECC).

To obtain a representative sample of each blackberry product, three different bottles of each wine and elixir were collected and the contents of all three bottles were mixed prior to analysis. All samples were stored in the dark at 2-8 °C until analysis.

Chemicals and reagents

Phenolic acid standards of gallic acid, caffeic acid, chlorogenic acid and *p*-coumaric acid were obtained from Sigma-Aldrich (Steinheim, Germany), while a phosphorus standard solution (concentration 1000 ± 0.002 mg/dm^3) was provided by AccuTraceTM (New Haven, CT, USA). Standard solutions of elements (concentration 1000 ± 0.002 mg/dm^3) for flame atomic absorption spectroscopy (FAAS) and flame atomic emission spectroscopy (FAES) were supplied by Panreac (Barcelona, Spain), while the mixed graphite furnace atomic absorption spectrometry (GFAAS) standard and matrix modifiers were purchased from Perkin Elmer (Shelton, CT, USA).

Lanthanum (III) chloride heptahydrate (99.9%) used as molecular suppressor was purchased from Roth (Karlsruhe, Germany) and nitric acid (TraceSelect, for trace analysis, ≥ 69.0%) used as reagent and detergent was supplied by Sigma-Aldrich (St. Louis, USA). Acetonitrile, methanol, ethanol and glacial acetic acid (all HPLC grade) were provided by Carlo Erba (Rodano, Italy), while sulfuric acid (96.0% (w/w), density 1.84 g/cm^3, p.a.), fuming hydrochloric acid (36.5% (w/w), density 1.18 g/cm^3, p.a.) and phosphoric acid (85% (w/w), density 1.70 g/cm^3, p.a.) came from Kemika (Zagreb, Croatia).

Folin-Ciocalteu's phenol reagent, ferric chloride anhydrous, potassium dihydrogen phosphate, potassium chloride and sodium acetate anhydrous were supplied by Fluka (Buchs, Switzerland). Potassium ferricyanide, trichloroacetic acid and butylated hydroxyanisole (BHA), as synthetic antioxidants, were purchased from HiMedia (Mumbai, India). DPPH (2,2-diphenyl-1-picrylhydrazyl, 95%) and ABTS (2,2'-azino-bis-[3-ethylbenthiazoline-6-sulfonic acid]) in the crystallized diammonium salt form were purchased from Sigma-Aldrich (Steinheim, Germany) as free radical sources. Sodium carbonate anhydrous, potassium persulfate and dipotassium phosphate were provided by Merck (Darmstadt, Germany). Kjeltabs Cu/3.5 (3.5 g potassium sulfate and 0.4 g copper sulfate pentahydrate) used as catalyst was supplied by Foss Tecator (Hoeganaes, Sweden). Ammonium sulfate, ammonium heptamolybdate tetrahydrate, boric acid, sodium hydroxide, bromocresol green and methyl red as indicators and other basic chemicals were supplied by Kemika (Zagreb, Croatia). Other reagents used in this work were ofanalytical reagent grade or higher purity. Double deionized 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 for all experiments.

Analytical methods

pH determination

The pH was measured using a digital pH meter MP 225 with a combined pH electrode INLAB 413 (Mettler Toledo, Switzerland) according to the Organisation Internationale de la Vigne et du Vin (OIV) reference method (OIV, 2008).

Determination of total polyphenols - Folin-Ciocalteu index (TPH)

The total polyphenol concentration in blackberry wines and elixirs was determined spectrophotometrically as described by the OIV (OIV 2008) using the reagent Folin-Ciocalteu. After an incubation period of 2 h at room temperature, the absorbance of the resulting blue colouration was measured at 760 nm. Gallic acid was used as a standard. Results were determined using a gallic acid standard calibration curve and expressed as gallic acid equivalents (mgGAE/dm^3) (Amidžić Klarić et al., 2011b; Amidžić Klarić et al., 2020).

Determination of total monomeric anthocyanins (ACY)

The total concentration of monomeric anthocyanins in blackberry wines and elixirs was determined using the pH differential method (Lee et al. 2005). The method is based on the fact that monomeric anthocyanin pigments change their colour and intensity reversibly at different pH values. All samples were diluted with two different buffers (potassium chloride buffer pH 1.0 and sodium acetate buffer pH 4.5), since at pH 1.0 the anthocyanins are in the "coloured" form of aromatic cations, whereas at pH 4.5 the "colourless" carbinol form predominates. Absorbance was measured simultaneously at 510 nm and 700 nm, and the difference in absorbance was proportional to the concentration of anthocyanins in the samples. ACY values were expressed as malvidin-3-glucoside equivalents (mgM3GE/dm^3). For malvidin-3-glucoside, a molar extinction coefficient of 28000 dm^3/(cm mol) and a molecular weight of 463.3 g/mol were used (Amidžić Klarić et al., 2011b; Amidžić Klarić et al., 2020).

HPLC analysis of phenolic acids

Chromatographic separations were performed on a Dionex (Sunnyvale, CA, USA) HPLC system consisting of an ASI 100 automatic sample injector, a P680 pump system, a TCC-100 column oven, a UVD170S detector and Chromeleon 6.8 software. Samples were filtered through Minisart RC4, 0.45 μm filters (Sartorius, Goettingen, Germany), which did not retain any of the analytes. The phenolic acids studied were separated on a Lichrospher 100-RP18 column (250 × 4 mm, 5 μm; Agilent Technologies, Santa Clara, CA, USA) with an appropriate precolumn. The chromatographic conditions were a modification of those of Gambelli and Santaroni (2004). Samples were stored in amber vials at 4 °C in an autosampler, and the injection volume was 20 μL. The mobile phase consisted of acetic acid:water (5:95) (eluent A) and acetonitrile (eluent B). The gradient elution was programmed as follows: 0-30 min, 0-80% B; 30-33 min isocratic 80% B. The elution was performed at a flow rate of 1 cm^3/min and the column was thermostatically controlled to a temperature of 30 °C. Absorbance during a chromatographic run was detected in the spectral range 200-600 nm. The detection wavelength for each analyte was as follows: 280 nm (gallic acid), 313 nm (*p*-coumaric acid) and 323 nm (chlorogenic acid and caffeic acid) (Amidžić Klarić et al., 2011b).
Quantitative analysis was performed in triplicate using external calibration curves. The proposed method was validated in terms of linearity, sensitivity, repeatability and average precision in accordance with the guidelines of the International Conference on Harmonization (ICH) (ICH, 2005).

Spectrophotometric methods

Spectrometric measurements were performed using the Lambda 25 UV-Vis spectrophotometer (Perkin-Elmer, Waltham, MA, USA). Absorbance was determined using a quartz cuvette with 10 mm path length (Perkin-Elmer, Waltham, MA, USA), while data were recorded using UVWINLAB (version 2.85.04) spectroscopy software (Perkin-Elmer, Waltham, MA, USA).

DPPH method

The ability of the studied blackberry wines and elixirs to scavenge the "stable" DPPH free radical was investigated using a slightly modified method of Hatano et al. (1988). Briefly, blackberry products were diluted with methanol. Then, an aliquot of 4 cm^3 of various concentrations of the sample and 0.5

cm^3 of 1 $mmol/dm^3$ of a methanolic solution of DPPH was added to the test tube. The mixture was shaken for 15 s and then allowed to stand at room temperature for 30 min. The absorbance of the resulting solution was read at 517 nm. Instead of pure methanol, a methanolic solution of 2 mg BHA dissolved in 4 cm^3 methanol with 0.5 cm^3 of the DPPH solution was used for background correction (Amidžić Klarić et al., 2011b; Amidžić Klarić et al., 2020).

ABTS method

Spectrophotometric analysis of ABTS radical scavenging activity was determined according to the method of Re et al. (1999). The ABTS monocation radical solution was prepared by mixing equal volumes of a 7 $mmol/dm^3$ ABTS solution and a 2.45 $mmol/dm^3$ potassium persulfate solution, both in DDW. The mixture was stored in the dark at room temperature for 12 h before use and diluted to give an absorbance of 0.700 ± 0.025 at 730 nm with DDW. Then, an aliquot of 3 cm^3 of the blackberry product diluted in DDW at different concentrations and 0.5 cm^3 of the ABTS solution was added to the test tube. The reaction mixture was incubated for 30 min at room temperature and the absorbance was immediately measured at 730 nm. The percentage of inhibition was calculated for each concentration relative to the blank absorbance (DDW).

For DPPH and ABTS methods, IC50% values were calculated as the concentration of blackberry product required for 50% inhibition (Amidžić Klarić et al., 2011b; Amidžić Klarić et al., 2020).

Reducing power assay (RPA)

The RPA was estimated based on the method described by Oyaizu (1986) and Yen and Chen (1995) with some slight modifications. Briefly, 0.1-1.0 cm^3 of each sample tested was diluted with DDW. Then, 1.0 cm^3 of the prepared aliquot, 2.5 cm^3 of a 0.2 mol/dm^3 phosphate buffer (pH 6.6) and 2.5 cm^3 of a 1% (w/v) solution of potassium ferricyanide were added to the test tube. The resulting solution was vortex mixed for 15 s and then incubated in a water bath at 50 °C for 20 min. Subsequently, 2.5 cm^3 of a 10% (w/v) trichloroacetic acid solution was added to the test tube and the mixture was centrifuged at 1750 x g for 10 min. A 2.5 cm^3 aliquot of the supernatant was combined with 2.5 cm^3 of DDW and 0.5 cm^3 of a 0.1% (w/v) solution of ferric chloride. The reaction mixture was shaken vigorously. The absorbance was recorded at 700 nm against the reagent blank. Increased absorbance of the reaction mixture indicates greater reducing power (Amidžić Klarić et al., 2011b; Amidžić Klarić et al., 2020).

Determination of total nitrogen

The Kjeldahl method is used to determine the total nitrogen content in blackberry products (OIV, 2008). The analysis was performed on the semi-automatic digestion system Kjeltec TM 2300 with Digestor 2006, Scrubber 2001 and Controller 2000 (Foss Tecator, Hoeganaes, Sweden) (Amidžić Klarić et al., 2011b; Amidžić Klarić et al., 2017).

Determination of total phosphorus

Total phosphorus concentration was determined spectrophotometrically using the vanadomolybdate reagent after mineralization of the sample (Zoecklein, 1995; OIV, 2008). Prior to analysis, each sample was digested using the wet ashing procedure with sulfuric and nitric acids. Briefly, the sample solution was appropriately diluted with DDW to obtain the required concentration. Then, a 10 cm^3 sample aliquot was transferred to a test tube and added to 10 cm^3 of vanadomolybdate reagent. The mixture was shaken well for 15 s using a vortex mixer (ZX3, Velp scientifica, Usmate, MB, Italy) and then allowed to stand for 10 min at room temperature. Then, the absorbance of the resulting solution was measured at 430 nm. The results were determined using a standard phosphorus calibration curve and expressed in mg/dm^3 (Amidžić Klarić et al., 2017).

Determination of metals in blackberry products

Analysis of minerals, trace elements and heavy metals was performed using a Perkin-Elmer AAnalyst 100 atomic absorption spectrometer equipped with an HGA-800 graphite furnace (Perkin-Elmer, Waltham, MA, USA) and a deuterium background corrector. The concentrations of calcium, copper, iron, magnesium, manganese, potassium and zinc in the samples were analyzed by FAAS, while sodium concentration was measured by FAES. The concentrations of cadmium, chromium and cobalt were determined by GFAAS. A detailed description of the main analytical parameters for the determination of all metals in the studied blackberry products was described in previous works (Amidžić Klarić et al., 2011a; Amidžić Klarić et al., 2016). Immediately after opening, blackberry wine and elixir samples were filtered through 0.45 μm filters

(Sartorius, Goettingen, Germany) and analyzed in triplicate without pretreatment. All samples were stored in sealed plastic bottles at 2-8 °C in the dark (Amidžić Klarić et al., 2011a; Amidžić Klarić et al., 2016).

Statistical methods

All determinations were performed in triplicate and the obtained data are presented as means ± standard deviations. Relative standard deviation (RSD) is the calculation of precision in data analysis, and it is calculated by dividing the standard deviation of a group of values by the average of the values. p<0.05 was considered statistically significant and p<0.01 very significant. STATISTICA ver. 12 statistical package from StatSoft® (Tulsa, OK, USA) was used for data analyses.

Results and discussion

The acidity of blackberries changes during growth and ripening and, thereby affecting the acidity of blackberry products. The first indicator of wine acidity is pH, and generally table wines have a pH between 3.3 and 3.7 (Zoecklein, 1995). From the data in Table 1, it can be seen that the pH of the blackberry wines and elixirs analyzed ranged from 3.28 (BECC) to 3.61 (BWBH). The obtained results are in agreement with our previous research results for blackberry wine (Amidžić Klarić et al., 2017; Amidžić Klarić et al., 2011b) as well as with the results reported for wines produced from blackberries in other studies (Johnson and Gonzalez Mejia, 2012; Gao et al., 2012).

Phenols in blackberry products

Blackberry is considered a rich source of bioactive compounds, such as polyphenols. Table 1 shows the total polyphenol content of the studied blackberry products. It can be seen that the three samples studied have TPH over 2000 mg/dm^3, while only the BWBH sample has a much lower TPH value (1101 mg/dm^3). Moreover, this sample had ACY content below 10 mg/dm^3 (6.62 mg/dm^3) and it was lower compared to the other samples tested (14.11-23.94 mg/dm^3). This is consistent with the available published results (Amidžić Klarić et al., 2011a; Amidžić Klarić et al., 2020) and the results of other authors reporting TPH and ACY content (Kalkan Yildirim, 2006; Mudnic et al., 2010; Mudnic et al., 2012; Johnson and Mejia, 2012; Čakar et al., 2016). It should be noted that anthocyanins are the dominant group of plant pigments of blackberry fruit and their stability is influenced by a number of factors, such as temperature, light, oxygen, pH, metals, sugars, the concentration of anthocyanins themselves and their chemical structure (Castañeda-Ovando et al., 2009). Since all processes, from extraction to storage, affect the degradation of anthocyanins, these pigments are relatively unstable biologically active compounds. Regarding the obtained results, large variations of ACY content in blackberry products were observed in our study.

In recent decades, numerous research studies have been conducted to identify and determine individual polyphenolic compounds in foods. Blackberry fruits and their products contain a number of phenolic acids. In this study, the concentrations of four phenolic acids (gallic acid, chlorogenic acid, caffeic acid and p-coumaric acid) present in blackberry wines and elixirs were determined using the appropriate HPLC method; the representative chromatogram is shown in Figure 1.

Our previous studies have shown that gallic acid is one of the dominant polyphenolic compounds in blackberry wines. Based on the results obtained (Table 1), the blackberry wines analyzed had a higher concentration of this phenolic acid (133.18 and 147.83 mg/dm^3) than the products designated as elixir (87.55 and 101.87 mg/dm^3). It was also found that only the sample BECC contained gallic acid below 100 mg/dm^3. Our results are in general agreement with the results of previous studies of blackberry wines (Amidžić Klarić et al., 2011a; Amidžić Klarić et al., 2020).

Chlorogenic acid is one of the most abundant biologically active polyphenolic compounds in human diet. This hydroxycinnamic acid was detected in all samples (0.897-2.513 mg/dm^3) and only BWCC sample had chlorogenic acid content less than 1 mg/dm^3 (Table 1). Moreover, the results of the present study show that the content of chlorogenic acid in both blackberry product samples from Bosnia and Herzegovina (average: 2.3305 mg/dm^3) is in agreement with the results obtained for blackberry wines originated from two subregions of continental Croatia - Prigorje-Bilogora (average: 2.493 mg/dm^3) and Zagorje-Međimurje (2.448 mg/dm^3) (Amidžić Klarić et al., 2011a).

Caffeic acid occurs naturally in many plant foods and its concentration in the studied samples ranged in a rather narrow interval (3.016 - 3.931 mg/dm^3) when compared with the results of previously published papers (Amidžić Klarić et al., 2011a; Amidžić Klarić et al., 2020).

The available literature data show that p-coumaric acid, a hydroxy derivative of cinnamic acid, is present in the fruit and its products (Szajdek and Borowska, 2008). According to the results of this study, the concentration of this natural hydroxycinnamic acid in the studied blackberry products varied from 2.262 to 3.383 mg/dm^3.

Table 1 pH, total polyphenolic content, monomeric anthocyanins, individual phenolic acids, antioxidant activity and metals in blackberry natural products

Parameter	BWCC	BWBH	BECC	BEBH	RSD
pH	3.37 ± 0.01	3.61 ± 0.01	3.28 ± 0.02	3.36 ± 0.01	0.16 - 0.47
TPH (mg/dm^3)	2214 ± 95	1101 ± 30	2861 ± 142	2088 ± 42	1.99 - 4.98
ACY (mg/dm^3)	20.63 ± 1.38	6.62 ± 0.23	23.94 ± 1.33	14.11 ± 0.53	3.53 - 6.67
Individual phenolic acids					
Gallic acid (mg/dm^3)	133.18 ± 0.32	147.83 ± 1.24	87.55 ± 0.34	101.87 ± 1.23	0.24 - 1.20
Chlorogenic acid (mg/dm^3)	0.897 ± 0.008	2.148 ± 0.038	1.020 ± 0.026	2.513 ± 0.016	0.62 - 2.52
Caffeic acid (mg/dm^3)	3.838 ± 0.010	3.016 ± 0.038	3.931 ± 0.048	3.927 ± 0.091	0.26 - 2.31
p–Coumaric acid (mg/dm^3)	2.262 ± 0.003	3.194 ± 0.017	3.383 ± 0.010	2.419 ± 0.007	0.14 - 0.54
Antioxidant activity					
DPPH (mg/dm^3)	5.33 ± 0.26	5.10 ± 0.15	5.98 ± 0.11	5.53 ± 0.28	1.87 - 5.07
ABTS (mg/dm^3)	4.64 ± 0.06	7.93 ± 0.10	6.31 ± 0.10	6.02 ± 0.24	1.22 - 3.97
Biogenic elements and metals					
N (mg/dm^3)	115.88 ± 2.21	53.70 ± 2.30	81.54 ± 2.12	83.50 ± 3.37	1.90 - 4.28
P (mg/dm^3)	204.83 ± 0.41	38.52 ± 0.42	139.45 ± 0.28	355.62 ± 6.69	0.20 - 1.88
K (mg/dm^3)	1271 ± 11	1101 ± 10	1016 ± 1	1101 ± 2	0.10 - 0.89
Na (mg/dm^3)	27.08 ± 0.14	12.42 ± 0.22	185.87 ± 0.35	34.22 ± 0.20	0.19 - 1.77
Ca (mg/dm^3)	94.96 ± 0.63	97.46 ± 0.03	125.17 ± 0.81	132.97 ± 0.55	0.04 - 0.66
Mg (mg/dm^3)	309.63 ± 0.95	318.30 ± 0.75	292.90 ± 0.40	401.03 ± 1.81	0.14 - 0.45
Fe (mg/dm^3)	0.470 ± 0.009	0.350 ± 0.009	0.169 ± 0.009	0.509 ± 0.011	1.89 - 5.23
Mn (mg/dm^3)	14.85 ± 0.06	14.60 ± 0.09	0.87 ± 0.01	12.60 ± 0.01	0.05 - 0.63
Cu (mg/dm^3)	0.097 ± 0.002	0.007 ± 0.001	0.117 ± 0.003	0.132 ± 0.002	1.42 - 6.02
Zn (mg/dm^3)	0.578 ± 0.002	0.350 ± 0.003	0.124 ± 0.001	0.578 ± 0.004	0.42 - 0.84
Co (μg/dm^3)	8.13 ± 0.06	2.78 ± 0.32	5.18 ± 0.52	4.32 ± 0.46	0.76 - 11.53
Cr (μg/dm^3)	22.16 ± 0.35	13.21± 0.44	12.51 ± 0.18	13.13 ± 0.43	1.41 - 3.35
Cd (μg/dm^3)	5.84 ± 0.59	2.61 ± 0.06	0.51 ± 0.15	5.23 ± 1.09	2.38 - 28.43

Legend: No significant differences were found between the triplicates for all determinations. The results are expressed as mean ± SD (n = 3); BWCC – blackberry wine Coastal Croatia, BWBH – blackberry wine Bosnia and Herzegovina, BECC – blackberry elixirs Coastal Croatia, BEBH – blackberry elixirs Bosnia and Herzegovina; RSD – Relative Standard Deviation; TPH – total polyphenolic compounds (values are expressed as milligrams of gallic acid per litre of sample); ACY – total monomeric anthocyanins (values are expressed as malvidin-3-glucoside equivalents); DPPH – $IC_{50\%}$ values for DPPH method; ABTS – $IC_{50\%}$ values for ABTS method.

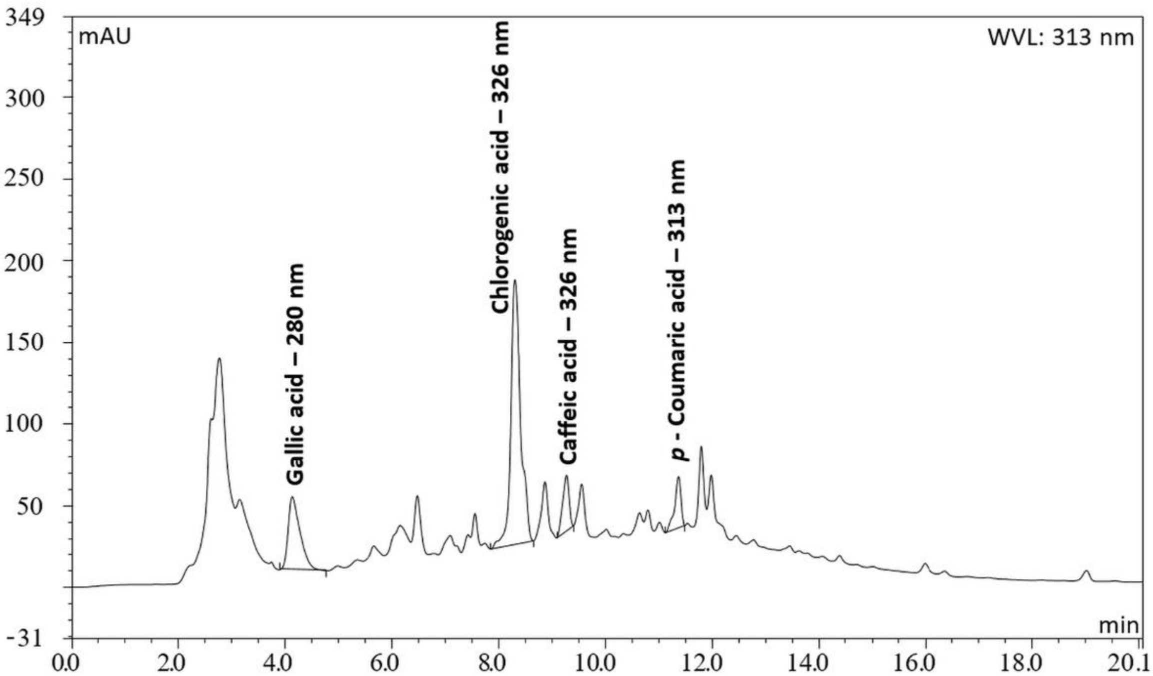

Figure 1. HPLC chromatogram of blackberry wine (sample BWCC) monitored at 313 nm for phenolic acids.

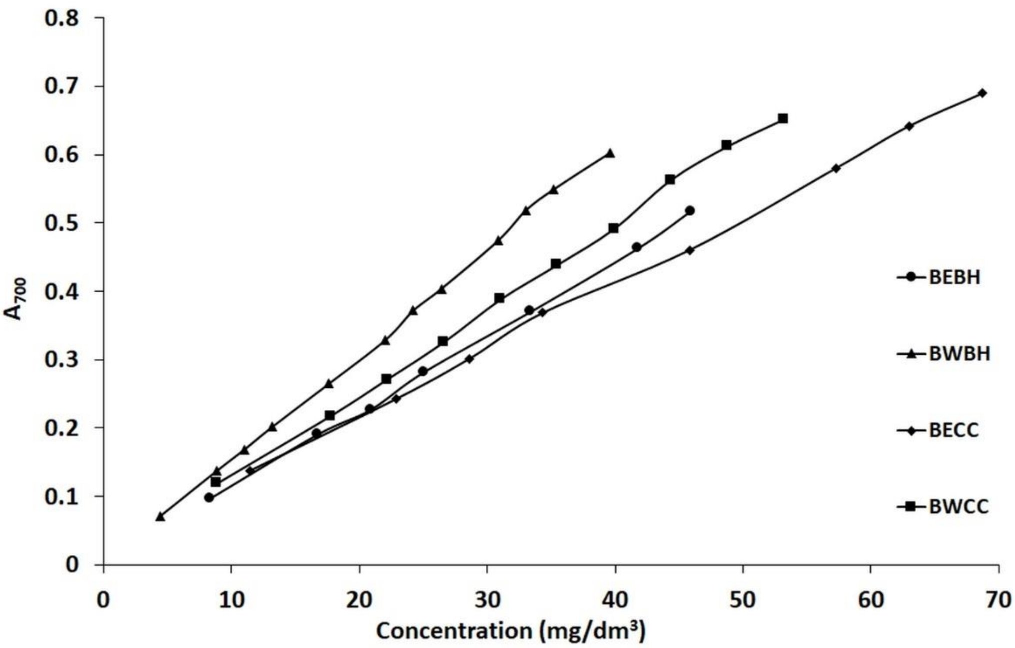

Figure 2. Antioxidant activity of investigated blackberry natural products expressed as reducing power.

Antioxidant activity of blackberry products

Intake of antioxidants, found in foods as a part of the normal diet of every population, is the most natural and uncomplicated way to meet daily needs.. Drinks are highly appreciated taken with meals or taken regardless of meals. Currently, various methods are used to evaluate the antioxidant activity of plant phenolic and other bioactive compounds. In this study, the antioxidant activity of blackberry samples is estimated by DPPH, ABTS and RPA methods. The DPPH radical scavenging activity of the studied blackberry samples was in a rather narrow range (Table 1) and in agreement with previously reported data (Piljac-Žegarec et al., 2007; Amidžić Klarić et al., 2011a; Ortiz et al., 2013), especially for the organic (IC50%=5.20 mg/dm^3) and conventional (IC50%=5.43 mg/dm^3) groups of blackberry wine from Croatia (Amidžić Klarić et al., 2020).

When evaluating the antioxidant activity of the studied products by the ABTS method, BWBH showed the weakest antioxidant activity, while the strongest effect had the BWCC sample (Table 1). The obtained results are in accordance with the previously published papers (Amidžić Klarić et al., 2011b; Amidžić Klarić et al., 2020).

Since antioxidants can be explained as reducing agents, the presence of these reductive substances in the blackberry samples causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. In the RPA assay, the yellow colour of the test solution changes to different shades of green and blue (Perl's Prussian blue) depending on the reducing power of the antioxidant samples. The reducing power increases according to the increase in absorbance. For this reason, the reducing power of a sample can serve as a significant indicator of its potential antioxidant activity. For comparison, the RPA of the blackberry samples was evaluated at a concentration of 40 mg/dm^3 (Figure 2).

The lowest absorbance of the resulting complex was measured in the sample BECC (0.413), while the highest was in the sample BWBH (0.615). This value is in agreement with the data reported for Croatian blackberry wine (Amidžić Klarić et al., 2011b; Amidžić Klarić et al., 2020).

Biogenic elements and metals in blackberry products

Biogenic elements such as nitrogen and phosphorus (i.e. nutrients) are essential for fruit growth, and blackberries contain these elements in varying amounts. Since these macronutrients are essential components of complex molecules, the total contents of these biogenic elements were determined by the wet ashing digestion method (mean recovery using the standard: 99.33% for N, 101.57% for P; n=3).

Total nitrogen values were determined from the sum of all nitrogenous compounds present in the studied blackberry products. The results obtained (Table 1) showed that the concentration of this biogenic element varied significantly in the wines studied (BWBH: 53.70 mg/dm^3; BWCC: 115.88 mg/dm^3), while it was uniform in the elixirs analyzed (BECC: 81.54 mg/dm^3; BEBH: 83.50 mg/dm^3). The obtained results are in agreement with our previous research findings for blackberry wines (Amidžić Klarić et al., 2011b; Amidžić Klarić et al., 2017).

It is important to emphasize that total phosphorus concentrations in the studied blackberry products were in a rather wide range (38.52-355.62 mg/dm^3). Moreover, the results of this study clearly show that only one sample, BWBH, had a total phosphorus concentration lower than 100 mg/dm^3. The obtained results are in agreement with our previous research findings (Amidžić Klarić et al., 2017).

Potassium is the dominant macromineral in blackberry wine, affecting wine pH and playing a significant role in wine stability. The potassium concentration was in a narrower range (1016 - 1271 mg/dm^3) in all samples tested than the interval (564-2014 mg/dm^3) determined in previous studies (Amidžić Klarić et al., 2011a; Amidžić Klarić et al., 2016).

It should be noted that sodium is widely distributed in food, and its daily intake from food is much higher than the amount required due to an unhealthy diet. High sodium consumption (2 g/day, equivalent to 5 g salt/day) and inadequate potassium intake (less than 3.5 g/day) contribute to hypertension and increase the risk of heart disease and stroke (WHO, 2020). The sodium concentration in the three samples was in a narrower range (12.42-34.22 mg/dm^3), with only BECC having a significantly higher value (185.87 mg/dm^3) than other samples. The Na content in blackberry wines is quite broad (3-213 mg/dm^3) and narrower than in grape wines (ND - 310 μg/cm^3) compared to other fruit wines (cherry, apple) (Alapez et al., 2014; Velić et al., 2018). If combining the results of our previous studies (Amidžić Klarić et al., 2011a; Amidžić Klarić et al., 2016) and the results obtained in this work for Na content (total number of samples: 34 wines and 2 elixirs), it can be seen that 88.89% of all samples had content below 150 mg/dm^3. Since the studied blackberry products are low sodium beverages and had a low Na/K ratio, these types of foods could be useful in the diet of individuals who need to limit sodium intake and maintain high potassium levels.

Calcium and magnesium are natural components of blackberry fruit and the concentrations of these macronutrients in blackberry products (Table 1) were in agreement with literature data (Amidžić Klarić et al., 2011a; Alapez et al., 2014; Amidžić Klarić et al., 2016). Nowadays, the daily intake of calcium and magnesium is covered by various formulated dietary supplements, which are commercially available in large numbers. It should also be noted that the analyzed blackberry products are good natural sources of these macronutrients.

Trace elements are minerals that are necessary in small amounts for the physiological functioning of the organism. Due to an unhealthy diet, deficiency of trace minerals is common all over the world, but especially in developing countries. On the other hand, the intake of these minerals in large amounts has toxic effects (Reilly, 2004) and the high concentration of these trace elements in the samples affects the instability of fruit wines. In the blackberry product samples studied, the concentrations of Cu, Zn and Fe were within a narrow range (Table 1) and below the internationally established maximum levels (Cu: 1 mg/dm^3; Zn: 5 mg/dm^3; Fe: 10 mg/dm^3 (white wine) and 20 mg/dm^3 (red wine)) (OIV, 2008).

The manganese content in wine depends partly on the uptake of minerals from the soil by plant roots and partly on anthropogenic sources. The results obtained showed that the concentration of this trace element in the samples studied varied from the minimum of 0.87 mg/dm^3 in the sample BECC to the maximum of 14.85 mg/dm^3 in BWCC. It was observed that the three studied products (BWCC, BWBH and BEBH)had higher manganese contents than Croatian blackberry wines (0.5-11.5 mg/dm^3) (Amidžić Klarić et al., 2011a; Alapez et al., 2014; Amidžić Klarić et al., 2016).

Cobalt is a bioessential element, predominantly as part of cobalamin and its main function in humans is based on its role in vitamin B12. On the other hand, this element is widely distributed in the environment at low concentrations. Humans are exposed to small amounts of cobalt by breathing polluted air or ingesting contaminated drinking water or food. The concentration of cobalt in the samples tested ranged very narrowly from 2.78 (BWBH) to 8.13 (BWCC) μg/dm^3. When compared with literature data, the results of this study showed that the examined blackberry products had similar levels of this element to Croatian blackberry wines (1.3-11.9 μg/dm^3) (Amidžić Klarić et al., 2011a; Amidžić Klarić et al., 2016) and significantly lower than cobalt concentrations in other types of wine (Pohl, 2007).

From a toxicological point of view, fruit wines and elixirs could be sources of certain elements that can be harmful to humans at sufficiently high doses, such as Cd, Cr and others. The cadmium content detected ranged from 0.51 μg/dm^3 to 5.84 μg/dm^3 in the blackberry products studied and was lower than the limits established by the OIV (0.01 mg/dm^3) for this non-essential heavy metal (OIV 2008). It is also important to mention that the tested blackberry samples contained chromium (12.51-22.16 μg/dm^3) in lower levels than the maximum allowed concentration (100 μg/dm^3) (Croatian Regulation No. 96/96 1996).

The concentrations of Co, Cd and Cr in the analyzed blackberry products were determined by GFAAS. One of the advantages of this technique is the relatively low detection and quantification limit, which is in the range of μg/dm^3 (or ppb) for most elements. Considering that the concentrations of Co and Cd in the analyzed blackberry wines were relatively low, the obtained results of the performed analysis can be accepted with the reported RSD values (Co: 0.76-11.53%; Cd: 2.38-28.43%).

Conclusions

To the best of our knowledge, the present study brings the first data on the content of polyphenols, antioxidant activity, nutrients, essential minerals and heavy metals in commercially available traditional blackberry products (i.e. wine and elixir) from Coastal Croatia and Bosnia and Herzegovina regions.
Consumption of the studied blackberry products, especially wine, could significantly increase the daily intake of antioxidants from food, which has often been associated with numerous health benefits in humans.
Considering the results obtained, traditional blackberry products, namely blackberry wine and blackberry elixir, could be considered safe for health and a good additional source of the essential minerals studied, such as manganese and potassium. Based on the results of this study combined with the results of our previous studies, both blackberry wines and elixirs could be classified as low sodium beverages.
The concentrations of the analysed undesirable toxic and potentially toxic metals in the investigated blackberry products were below the maximum permissible concentrations defined by various regulations for wines and fruit wines.

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