DOI: 10.46793/SBT28.405P

ANTIOXIDANT PROPERTIES AND BIOLOGICAL ACTIVITY OF FRUIT WINES

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Abstract: The fruit wines from blueberry were made by microvinification procedure. Wines were produced in the absence or presence of sugar and/or enzymatic preparation glycosidase (EPG). Selected phenolic acids were quantified using UPLC/MS-MS analysis. Total phenolic content (TPC) was determined by the Folin-Ciocalteu method. Also, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing ability of plasma (FRAP) methods were applied. The α-glucosidase inhibitory activity of blueberry wines was also investigated. Wines made with addition of sugar and EPG showed the best results. Phenolic profile and biological activity of blueberry wine depended from microvinification procedure which was used in the production.

Keywords: blueberry wine, phenolic compound, antioxidant activity, anti radical activity, α-glucosidase inhibitory activity

Introduction

Fruits and vegetables are essence part of balanced diet and their regular consumption (400 g every day), as fresh or processed may positively affect human health (Joshipura et al., 2001). Antioxidant compounds are responsible for health promoting effects of food, since they prevent development of chronic non-communicable diseases such as heart disease and diabetes mellitus (WHO, 2016). Fruits and their derived products are rich source of numerous naturally occurring compounds such as phenolic acids (hydroxybenzoic and hydroxycinnamic acid derivatives), flavonoids and anthocyanins (Robards et al., 1999;. Wang, 2003). The fruit wine is one of the products which is possible to make from various types of fruit. It is important to emphasize that during fruit processing active principles retain in the final product (Czyzowska and Pogorzelski, 2002). Berry fruit wines are

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especially known for their good antioxidant and radical scavenging properties (Johnson et al., 2011). It is also interesting to highlight the natural active principles from fruit, which display α-glucosidase inhibitory activity. The α-glucosidase represents enzyme, which is involved in the final step of the carbohydrate digestion. Natural active principles which have α-glucosidase inhibitory activity express much lower side effects then synthetic ones, and can be used in the control of postprandial hyperglycemia (Xiao and Hogger, 2015;McDougall et al., 2005).

The aim of this study was to show how microvinification procedure influences on the content of selected phenolic compounds and antioxidant, as well as on free radical scavenging properties of the wine. Also, α-glucosidase inhibitory activity of lyophilized fruit wine samples was evaluated.

Materials and methods

Blueberries (*Vaccinium myrtilus*) were obtained from the region of Rudnik mountain, Serbia. Microvinification procedure was conducted in two sets, and the first step in both sets was fruit disintegration. In order to inhibit growth of unwanted bacterial populations during fermentation 10 g of $K_2S_2O_5$ 100/kg was added in the fruit must. Total soluble solids (expressed in °Brix) were measured in the fruit must of the first set. Aiming to increase total soluble solids of must up to 20.5°Brix, sugar was added in the second set. Within the aforementioned sets of the experiment, two subsets were performed. While the first sub-set included addition of 2 g/100 kg of enzymatic preparation glycosidase (EPG) (Enartis, Italy), the second one omitted its use. Both sub-sets were inoculated with pure culture of the selected commercial wine yeast (ICV D254, Lallemand, Canada (L), and Lievito Secco, Enartis, Italy (E)) both yeasts *Saccharomyces cerevisiae* strain) in the amount of 20 g/100 kg, respectively. Microvinification was conducted in barrels with pigeage system while alcohol fermentation was conducted on 20°C over the next 7 to 10 days. After fermentation, blueberry wine was separated from pomace by sedimentation, and kept at 12°C for the next six months, until further studies.

Total soluble solids (TSS, expressed in °Brix)and alcohol concentrationexpressed as volume (vol. %) were evaluated.

Solid-phase extraction (SPE) on Oasis HLB 6CC 200 mg cartridges (Waters, Milford, MA, USA) was applied to decrease the influence of the matrix during phenolics identification. UPLC/MS-MS analysis was performed using a Waters Acquity Ultra Performance H-Class System (Waters, Milford, MA, USA). Redox potential of the fruit wine samples was determined using the FRAP test (Benzie and Strain, 1996).The obtained results were expressed in mmol/L Fe^{2+}. Anti-DPPH radical activity of the fruit wine samples was evaluated as previously

described (Blois, 1958). The obtained results were expressed as a reciprocal value I (%) multiplied by 100. Total phenolic content (TPC) of the fruit wine samples was estimated by the Folin–Ciocalteu method using gallic acid as a standard (Woraratphoka et al., 2007). The results were expressed in mg/L of gallic acid equivalents (mg GAE/L).

Lyophilized wine samples were tested for α-glucosidase inhibitory activity using modified method (McCue et al., 2005). Acarbose was used as a positive control. The results were expressed as IC_{50} (μg/mL). The p value lower than 0.05, was considered as a significant.

Results and discussion

Physicochemical parameterTSS content can predict alcohol content in wine (Table 1).

After UPLC/MS-MS analysis of blueberry wine control samples were compared with those ones made with sugar and/or EPG (Table 2). Actually, the sugar content significantly affected the content of selected phenolic compounds ($p<0.05$). Higher sugar content before fermentation leads to more abundant alcohol content in the final product which improve the extraction of phenolic compounds ($p<0.05$).EPG liberates phenolics from the glycoside form. It ispossible to highlight that microvinification procedure significantly affects on the content of phenolic compounds. The highest content was found in the blueberry wine samples prepared with sugar and EPG. Furthermore, the samples prepared with sugar/without EPG were richer in phenolics than those without sugar and with or without EPG. Samples prepared without sugar/with EPG contained more phenolic compounds than Control. Different yeasts used in microvinification did not show any significance in content of phenolic compounds ($p>0.05$). The major phenolic acid of blueberry wines was chlorogenic acid (Table 2.). Hydroxycinnamic acid derivatives such as caffeic and *p*-coumaric acids were also identified which was according to literature data (Häkkinen et al., 1999; Zadernowski et al., 2005). Gallic acid was also detected in significant amounts.

Protocatechuic acid was the most abundant hydroxybenzoic acid derivative, as previously reported (Zadernowski et al., 2005). Additionally, Häkkinen et al. (1999) confirmed the presence of *p*-hydroxybenzoic acid in blueberries. Hydroxycinnamic were more abundant than hydroxybenzoic acid derivatives, as described before (Zadernowski et al., 2005). Epicatechin and catechin were also identified, as expected (Liwei et al., 2003). Blueberry wines were enriched with epicatechin (65.84μg mL-1) ($p < 0.05$). Such a finding is well supported by a Dutch study (Arts et al., 2000).

Table 1. TSS content of must and alcohol content in ine

Type of wine	Total soluble solids of must (°Brix)	Alcohol content in wine (Vol. %)
Control E.	14.67	7.97
+sugar− enzyme E.	18.48	9.81
−sugar+ enzyme E.	14.71	8.12
+sugar+ enzyme E.	18.75	10.51
Control L.	14.57	8.27
+sugar− enzyme L.	19.11	11.00
−sugar+ enzyme L.	14.77	8.45
+sugar+ enzyme L.	19.27	11.25

Control -sugar-EPG

Different experimental sets in microvinification showed the same trend for the antioxidants content assayed by TPC, FRAP and DPPH (Table 3.) just like UPLC/MS-MS analysis ($p<0.05$). The highest content of antioxidants was detected in the sample with sugar and EPG, while the lowest was in those without sugar and without EPG. Different yeasts used in microvinification did not show any significance for this three parameters ($p>0.05$). The FRAP and DPPH depended on the cumulative (synergistic) effect of various compounds present in fruit wines.

Table 2. The content of phenolic compounds in blueberry wine (μg/ml)

Type of wine	1	2	3	4	5	6	7	8
Control E.	725.15	92.47	1.71	54.12	75.23	2.27	32.18	42.51
+sugar - enzyme E.	775.57	120.51	3.41	65.43	114.72	4.82	42.83	60.45
−sugar +enzyme E.	738.23	97.81	2.31	52.31	79.51	3.88	37.81	41.27
+sugar +enzyme E.	797.47	127.87	4.27	72.71	118.89	7.81	47.17	63.42
Control L.	727.15	90.52	1.85	55.71	77.12	2.37	30.45	41.23
+sugar −enzyme L.	777.57	122.31	3.17	67.57	115.84	4.62	43.77	61.57
−sugar +enzyme L.	736.43	96.43	2.47	55.43	81.51	4.17	35.76	44.52
+sugar +enzyme L.	801.23	125.91	4.35	71.48	120.45	8.21	48.64	65.17

1-Chlorogenic acid; 2-Caffeic acid; 3-*p*-Coumaric acid; 4-Gallic acid; 5-Protocatehuic acid; 6-*p*-Hydroxybenzoic; 7-Catechin; 8- Epicatechin

The results for TPC, FRAP and DPPH depended from alcohol content which improved the extraction of phenolic compounds leading to the enhanced TPC, antioxidant and antiradical potential of the final product. EPG also contributed to more profound antioxidant and antiradical potential. Another study by Vasantha Rupasinghe and Clegg (2007) showed significantly profound redox potentials for blueberry which is in line with our findings.

Table 3. FRAP, TPC, DPPH and α-glucosidase inhibition values for blueberry wine samples

Type of wine	FRAP (mmol L^{-1} Fe^{2+})	TPC (mg GAE L^{-1})	DPPH (IC_{50})	α-glucosidase inhibition (μg/ml)
Control E.	68.15	2234.45	1.57	45.26
+sugar −enzyme E.	77.18	2473.57	1.35	41.52
−sugar +enzyme E.	72.65	2317.21	1.53	34.18
+sugar +enzyme E.	83.21	2581.37	1.27	31.27
Control L.	69.23	2245.51	1.55	43.81
+sugar −enzyme L.	76.81	2489.83	1.39	32.25
−sugar +enzyme L.	71.43	2375.53	1.52	40.45
+sugar +enzyme L.	82.65	2595.17	1.31	30.15

The α-glucosidase inhibitory activity showed the same trend as UPLC/MS-MS, TPC, antioxidant and antiradical analysis ($p<0.05$) (Table 3). Values for IC_{50} of fruit wines were obtained from sigmoidal-shaped inhibition curves. The results indicated that our samples possess good α-glucosidase inhibitory activity (Table 3). Such findings are supported by the study of blueberry extracts which displayed a significant α-glucosidase inhibitory activity (McDougall et al., 2005).

Conclusion

In summary, it is possible to highlight blueberry wine as an important source of naturally occurring antioxidants, which exhibit health-promoting properties. The findings also indicated that blueberry wine inhibit α-glucosidase, and may be potentially used as a functional (medicinal) food in the

prevention of diabetes mellitus and other chronic diseases. Hydroxybenzoic and hydroxycinnamic acid derivatives jointly with other active principles – both phenolics and non-phenolics are responsible for beneficial health effects of fruit wine. Moderate consumption of blueberry wine may be recommended as a part of healthy (well-balanced) diet.

Acknowledgement

This research was funded by the Ministry of Science, Technological Development and Innovation, Republic of Serbia through Grant Agreement with University of Belgrade-Faculty of Pharmacy No: 451-03-47/2023-01/ 200161.

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