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Influence of blackberry juice addition on mead fermentation and quality

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Abstract:
Introduction. Mead, one of the oldest alcoholic beverages that man consumed is obtained by fermentation of honey solution, and contains from 8 to 18% vol. ethanol. Honey can be considered as an excellent source of carbohydrates for the fermentation process, but low concentrations of other substances in the honey can slow down the process. Blackberry (*Rubus fruticosus* L.) contains dietary fibers, vitamin C (ascorbic acid), vitamin A, vitamin E, potassium and calcium, along with the phenolic metabolites that are a source of possible health benefits.
Study objects and methods. In this study was investigated the influence of blackberry juice addition on mead fermentation process, chemical composition and antioxidative activity. Dynamics of the fermentation process were controlled based on weighing the flasks in time on a scale every 24 h throughout the alcoholic fermentation. At the end of fermentations, oenological parameters of mead: dry matter content, pH, volatile acidity and ethanol content and reducing sugars. For the determination of antioxidative capacity the content of total phenolics, total flavonoids and total flavonols were measured and two tests were performed: DPPH and ABTS.
Results and discussion. Addition of blackberry juice had a positive effect on fermentation dynamics (almost 25% higher rate of fermentation than in control samples), and improved all physicochemical characteristics and composition of resultant meads. Also, meads with the addition of blackberry juice had a significantly higher concentration of total phenolics, total flavonoids and total flavonols and significantly stronger antioxidative properties compared to the control meads without juice addition. The highest total phenolics, total flavonoids and total flavonols content was determined in the mead with the maximum addition of blackberry juice (B20W): it reached 490.88, 50.34 and 62.57 μgQE·mL^{-1}, respectively, and was 6-fold higher for total phenolics and total flavonoids content, and 10-fold higher for total flavonols content than in the mead without juice addition (CW). The strongest antioxidative activity was determined in the B10W mead; it accounted for 6.98 μgTE·mL^{-1} (DPPH assay) and 0.65 μgTE·mL^{-1} (ABTS assay), what was 1.5-fold and 3-fold higher, respectively, than in the mead without juice addition (CW).
Conclusion. The conducted study demonstrated that the use of blackberry juice influenced the course of fermentation of meads as well as their physicochemical and antioxidative properties (positive effect on fermentation dynamics – almost 25% higher rate of fermentation than in control samples, and improvement of all physicochemical characteristics and composition of resultant meads).

Keywords: Fruit, blackberry, beverages, fermentation rate, mead, antioxidant activity, kinetic model

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INTRODUCTION

Mead, one of the oldest alcoholic beverages that man consumed is obtained by fermentation (similar to the process of making white wine) of honey solution, and contains from 8 to 18% vol. ethanol [1]. Honey can be considered as an excellent source of carbohydrates for the fermentation process, but low concentrations of other substances in the honey (e.g. nitrogen) can slow down the process. Therefore, there is a need for adding nitrogen and various additives such as fruit pulps or juices, citric acid, coconut milk blend etc., but their addition should not hide the smell and taste of honey [2–5]. These additives are used to improve fermentation rates, alcohol yields and sensory characteristics of meads [1].

To enhance the character and complexity of meads, a variety of fruits, vegetables, herbs, or spices (ginger, cardamom, cloves, thyme, rosemary, bay leaves, sage, parsley, fennel, cinnamon, nutmeg, lemon or orange peels, among others) may be added. Fruits and their pulps have been highly recommended because of their richness in carbohydrates, fibers, minerals, vitamin C, carotenoids, phenolic and sulfuric substances. Also, their antioxidant action can help to maintain a balance between production and elimination of reactive oxygen species and other related compounds, thereby attenuating free radical-induced damage to cells [4]. Blackberry (*Rubus fruticosus* L.) fruit is traded globally due to its delicious taste, pleasant flavor and excellent nutritional profile. These fruits are consumed fresh or processed to make food products such as jam, wine, tea, ice cream, desserts, seedless jellies and bakery products. Blackberries contain dietary fibers, vitamin C (ascorbic acid), vitamin A, vitamin E, potassium and calcium, along with the phenolic metabolites that are a source of possible health benefits [6]. The content of biologically active compounds in meads and their antioxidative activity depend on many factors: type of honey, heat processing of wort, parameters of fermentation process, the type of used herbs, spices, fruits, etc [7–9]. Several structured and unstructured mathematical models have been developed in order to describe the fermentation reaction. A number of studies have been done on kinetic modeling of ethanol as regards its fermentation time. Fermentation rate i.e. kinetic of ethanol production can be described by modified Gompertz equation defined as in Eq. (1) [10, 11]:

$$P = P_{\mathrm{m}} \cdot \exp\left\{-\exp\left[\frac{r_{\mathrm{p,m}} \cdot \exp(1)}{P_{\mathrm{m}}}\right](t_{\mathrm{l}} - t) + 1\right\} \quad (1)$$

where P_m is the maximum ethanol content, g; $r_{p,m}$ the maximum rate of ethanol production, g·day^{-1}; t is duration of fermentation, days; and t_l is the lag time, days. In the available literature there is insufficient data on the production of Blackberry mead, so the objective of this study was to determine the effect of Blackberry juice addition to honey wort on fermentation performances, chemical composition, content of phenolic compounds and on the antioxidative properties of the produced meads.

STUDY OBJECTS AND METHODS

Chemicals and equipment. All chemicals used in this study were of analytical grade. The equipments were as follows: scales (H54AR, Mettler-Toledo, Columbus, USA and PFB 1200-2, KERN & SOHN, Balingen, Germany), hand blender (MSM7150, Bosch, Stuttgart, Germany), ultrasonic bath (U300, Ultrawave Limited, Cardiff, UK), magnetic stirrer (ARE, Velp Scientifica, Usmate, Italy), vortex (ZX3, Velp Scientifica, Usmate, Italy), rotary evaporator (Devarot, Elektromedicina, Ljubljana, Slovenia), spectrophotometers (6315 Jenway, Cole-Palmer, Staffordshire, UK and Spectronic 1201, Milton Roy, Ivyland, USA), pH meter (HI-2211, Hanna Instruments, Smithfield, USA), waterbath (Wisecircu, J.P. Selecta, Abrera, Barcelona, Spain), refractometer (Leica Abbe Mark II, Reichert Technologies, Depew, USA), conductivity meter (HA-2315, Hanna Instruments, Smithfield, USA), Bunsen burner, muffle furnace (Vims elektrik, Novi Sad, Serbia).

Samples. Blossom honey and blackberry fruit (*Rubus fruticosus* L.) from Thornfree cultivar needed for this study were aqiured on September 2016 in Mrkonjić Grad, municipality Mrkonjić Grad, Bosnia and Herzegovina. They were transported to the laboratory (being protected from the sunlight) and in the laboratory they were store in the dark at 2–4°C during 48 h.

Physicochemical analyses of honey. In accordance with the requirements established in Bosnia and Herzegovina legislation, the characteristics and satisfactory quality of the honey were assured through an analysis of the following parameters: moisture content, diastase activity, HMF content, acidity, reducing sugars, saccharose, electrical conductivity and ash content as described by Official Methods of Analysis [12, 13]. The pH was measured with a pH meter of honey dissolved in bidistilled water.

Honey must preparation. Blossom honey was stirred with water in the ratio 1:5 (honey/water). The resultant wort was pasteurized at 65°C for 10 min with regular stirring and skimming off the scum then cooled and poured into fermentation flasks. Blackberry fruit was pressed through a laboratory press to obtain juice that was further used in the study. Resultant juice was also pasteurized at 65°C for 10 min, cooled and poured into fermentation flasks in amounts required for this study. Afterwards, pH values of the wort and juice were corrected to 3.7–4 and four samples were prepared: control wort (CW) and three worts with added blackberry juice in the amount of 5% (B5W), 10% (B10W) and 20% (B20W) of fermentation wort volume. Into all variants yeast energizer VitaFerm® Ultra F3 (Erbslöh, Geisenheim, Germany) was added in amount of 0.1 g·L^{-1}. Next, commercial yeast Vulcaferm (Vulcascot, Wien, Austria), a specifically selected dry yeast strain of *Saccharomyces cerevisiae*, was rehydrated in distilled water at 35–40°C during 30 min and added in the amount of 0.6 g·L^{-1} of wort. The process of alcoholic fermentation was conducted at 25°C for 10 days. All fermentations were carried out in triplicate using a system that consisted of 250 mL flasks containing 180 mL of wort mixture and fitted with an airlock used to release CO_2 produced during fermentation. Dynamics of the fermentation process were controlled based on weighing the flasks in time on a scale every 24 h throughout the alcoholic fermentation.

General oenological parameters. At the end of fermentations, oenological parameters of mead: dry matter content, pH, volatile acidity and ethanol content

Table 1 Physicochemical parameters of honey

Tested parameter	Bosnia and Herzegovina legislation [12]	Honey
Moisture content, %	Not more than 20	19.40 ± 0.10
Diastase activity, Schade scale	Not less than 8	27.00 ± 0.50
HMF content, mg·kg^{-1}	Not more than 40	1.795 ± 0.005
Acidity, mmol·L^{-1}	Not more than 50	41.50 ± 0.50
Reducing sugars, g·100 g^{-1}	Not less than 60	74.80 ± 0.26
Saccharose, g·100 g^{-1}	Not more than 5	1.410 ± 0.003
Electrical conductivity, mS·cm^{-1}	Not more than 0.8	0.354 ± 0.005
Ash content, g·100 g^{-1}	Not more than 0.6	0.15 ± 0.01
pH	n.r.*	3.57 ± 0.01

*n.r. – not regulated

were determined according to standard methods and reducing sugars by 3,5-dinitrosalicylic acid method [14, 15].

Determination of total phenolic content. The total phenolic content in meads was measured spectrophotometrically according to the Folin-Ciocalteau method, as described by Wolfe and Liu [16]. Gallic acid standard in different concentrations (0–500 μg·mL^{-1}) was used to obtain a standard calibration curve. Results were expressed as total phenolics equivalent to gallic acid (μgGAE·mL^{-1}).

Determination of total flavonol content. The total flavonol content in meads was measured using the method of Kumaran and Karunakaran [17]. Results were expressed as flavones equivalent to quercetin (μgQE·mL^{-1}).

Determination of total flavonoid content. The total flavonoid in meads was measured using the method of Ordoñez *et al.* [18]. Results were expressed as flavonoids equivalent to quercetin (μgQE·mL^{-1}).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The antioxidant activity of meads against stable DPPH radical was determined by the method of the Liyana-Pathiranana and Shahidi [19]. Trolox (1–10 μg·mL^{-1}) was used as reference standard. The results were expressed in μgTrolox Equivalent·mL^{-1} (μgTE·mL^{-1}).

2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging assay. The test was performed as described by Re *et al.* [20]. Trolox (0.1–5 μg·mL^{-1}) was used as reference standard. The results were expressed in μgTrolox Equivalent·mL^{-1} (μgTE·mL^{-1}).

Table 2 Kinetics of the fermentation process of meads (g-cumulative mass of produced ethanol)

Sample	Day				
	3	6	8	9	10
CW	3.085	7.040	8.296	8.767	9.168
B5W	5.314	9.723	10.440	10.857	11.014
B10W	5.697	9.966	10.405	10.752	10.875
B20W	5.593	9.723	10.053	10.404	10.457

Statistical analysis. All tests were performed in triplicate and the results were expressed as means ± standard deviation. Variance analysis (ANOVA) was applied to test significant differences between mead samples. Tukey's test was used to identify differences between mean values obtained in meads ($P \leq 0.05$). Characteristic kinetic parameters of alcoholic fermentation were obtained by fitting the measured values of ethanol production into a modified Gompertz equation, performing nonlinear regression analysis. The statistical analysis of the developed mathematical relations was done applying linear regression analysis and Fisher's statistical tests.

RESULTS AND DISCUSSION

Based on the results presented in Table 1, the characteristics and quality of the honey were in agreement with the requirements established by Bosnia and Herzegovina legislation, and that the tested sample represents a good starting raw material for the production of mead [12].

The pH of honey was 3.57. This parameter is useful in quality evaluations because it influences the HMF formation rate, honey texture, stability and shelf-life, and may indicate honey fermentation or adulteration caused by the bees themselves. The fermentation of the analysed meads spanned for 10 days (Table 2).

Kinetics of the fermentation process of meads were determined based on changes in the weight of fermentation samples in time and expressed as the cumulative mass (g) of produced ethanol per day. The kinetics of ethanol production during fermentation of analysed samples were presented in Fig. 1.

The graphs in Fig. 1 show quite well overlapping of fitted curves with experimentally determined data. The correlation coefficient (R^2), as measurement of the fit's goodness, is 0.980 for CW sample and 0.997 for all other samples (BW5, BW1 and BW20), i.e. the samples with added blackberry juice. As it can be seen from the graphs, mass of produced ethanol rapidly increases after lag phase of fermentation process, corresponding to the exponential growth of the yeast cells.

The characteristic kinetic parameters of developed equations are summarized in Table 3, and compared to the corresponding parameters calculated from the experimental data (Table 2). The predicted values of the maximum mass of ethanol produced (P_m) correspond quite well to the actual values. Maximum rates of ethanol production ($r_{p,m}$), calculated from the experimental data, were being lower than predicted values, which can be explained by the fact that conducted experiments did not involve measurements of ethanol production during the lag phase. According to the developed equations this phase last from 1.6 to 1.9 days. Cuenca *et al.*, reported the lag phase in the mead fermentation being from 5 to 65 h [10].

According to the data in Table 3 and curves in Fig. 1, samples with the blackberry juice added (BW5, BW10 and BW20) show almost identical mathematical dependence of the ethanol production on time. Therefore, one can use single equation, with mean values of characteristic parameters, to describe production of ethanol during fermentation of mead with added blackberry juice in different amounts. Also, addition of the blackberry juice in the mead-making process had a positive effect on fermentation kinetic giving a rise to almost 25% higher rate of fermentation than in control samples (CW). The rate of fermentation depends on concentration of different inhibitors such as: ethanol, acetic acid, fatty acids (hexanoic, octanoic, decanoic acid), proteins (enzymes), furfural, hydroxymethylfurfural etc. [21]. The inhibitors interact synergistically with high osmotic pressure and the increasing concentration of ethanol during fermentation. Adamenko *et al.* investigated the influence of added juices from Cornelian cherry cultivars on mead fermentation and showed that juices addition caused CO_2 emission to be 10% higher than in control samples (without added juice) after 5 days of the fermentation process [3].

Based on the results shown in Table 4, it can be noticed that introduction of blackberry juice in mead fermentation improved all physicochemical characteristics and composition of resultant meads. Resultant dry matter contents were not too high, and among different samples were small differences. In sample CW, the dry matter content was the highest and in samples B5W and B10W the lowest (4.88 and 3.85%, respectively) which was an indicator, together with the reducing sugar content, that the fermentation process was almost at the end. According to the results of the content of residual sugar, it was obvious that the residual dry matter comes from other substances such as disaccharides (saccharose, maltose, isomaltose), trisaccharides, tetrasaccharides, glycerol, etc. [22]. Monitoring pH and acidity after honey-must

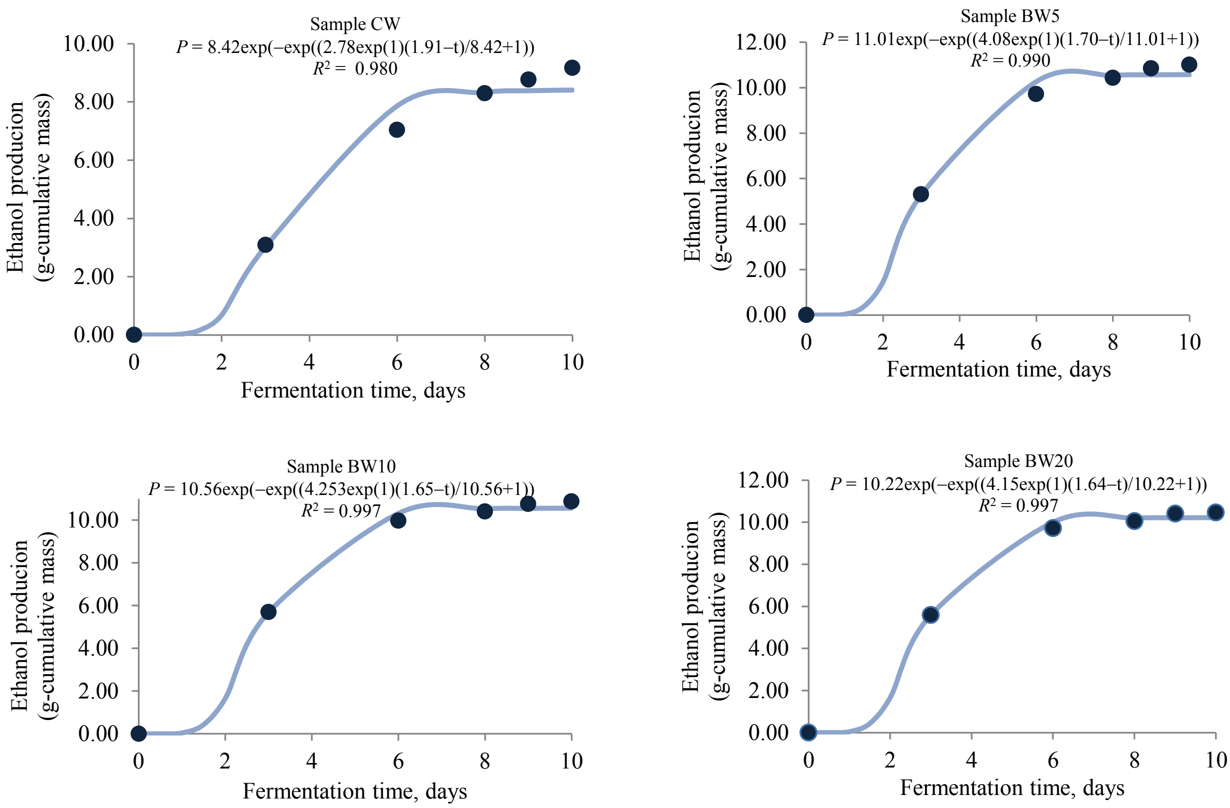

Figure 1 Ethanol production kinetic and results from fitting the experimental data into a modified Gompertz equation (solid line – fitted curve, symbol ● – experimental data)

Table 3 Kinetic parameters of the developed mathematical models and corresponding experimental data

Sample	P_m, g-cummulative mass	$r_{p,m}$, g·day^{-1}	t_l, day	R^2
CW	8.42 9.17*	2.78 1.33*	1.91 –	0.980
BW5	10.58 11.01*	4.08 1.77*	1.70 –	0.990
BW10	10.56 10.88*	4.25 1.90*	1.65 –	0.997
BW20	10.22 10.46*	4.15 1.86*	1.64 –	0.997

* measured values

preparation and during fermentation were important issues to prevent premature fermentation arrest and incomplete sugar breakdown. Acetic acid and succinic acid produced during fermentation by yeasts lead to an increase in the content of unsaturated fatty acids, which can cause slowing or even stopping fermentation. The lowest pH value and acidity were measured in sample B20W and the highest in sample CW (3.07 and 3.30, respectively), indicating that the samples with added blackberry juice had lower pH values compared to the control sample (CW), which could be due to the naturally low pH value of added blackberry juice. Acidity plays a significant role in alcohol beverages as it has a direct impact on their taste and stability [8]. In addition, excessive decrease in the pH value may contribute to reduced fermentation performance yield of a yeast strain [1]. The pH values of meads analyzed in this study were similar to those reported by Adamenko *et al.* and Akalin *et al.* but lower than Kawa-Rygielska *et al.* [3, 8, 23].

The titrable acidity increased during fermentation from 3.5 g·L^{-1} in the honey-must (data not shown), to 5.39 g·L^{-1} (B20W) in the final mead. Samples with added blackberry juice had higher titrable acidity than CW which could be due to the naturally high titrable acidity of added blackberry juice. Our results were pretty low compared to other authors [8, 22, 24]. Another important indicator of mead quality is volatile acidity. The production of acetic acid, by far the most abundant volatile acid, can have a dramatic effect on the quality of the final product. In addition to undesirable aromas, high levels of acetic acid were toxic to yeast and can led to stuck alcoholic fermentations. The volatile acidity in our study ranged from 400 to 660 mg·L^{-1} of acetic acid and was lower than the values reported by other authors [1, 3, 8, 23]. Reduction of volatile acidity in samples with blackberry juice was probably due to decrease of acetic acid synthesis by cells of *Saccharomyces cerevisiae* due to change in conditions or by a limitation of stress factors [24]. In our study the addition of blackberry juice improved the concentrations of ethyl alcohol from 6.93% vol. in CW to 7.98% vol. in B10W. The effect of meads supplementation on ethanol content, was studied by various authors. Pereira *et al.* reported the highest ethanol concentration in the samples with coupled addition of vitamins and mineral salts [1]. Kawa-Rygielska *et al.* determined the effect of fruit additives such as syrup from chokeberry or grape seeds, and herbal in the form of a dandelion syrup on the course of the fermentation process of "trójniak" before and after aging and reported the highest ethanol content in samples with added grape seeds powder or sugar syrup from chokeberry fruits, but lower in sample with added dandelion syrup [23]. In study conducted by Adamenko *et al.* it was demonstrated that ethanol production is affected by both the type of Cornelian cherry juice and the yeast strain used in mead manufacture: alcohol production by the SF yeast was 20 g·L^{-1} higher compared with the SM yeast, and more ethyl alcohol was produced in the sample with juice from red-fruit Cornelian cherry (MR) [3].

Study results demonstrate that the meads with the addition of blackberry juice had a significantly higher total phenolic, total flavonoid and total flavonol content and significantly stronger antioxidant activity compared to the control meads without juice addition (CW). The highest total phenolic, total flavonoid and total flavonol content was determined in the mead with the maximum addition of blackberry juice (B20W): it reached 490.88, 50.34 and 62.57 μgQE·mL^{-1}, respectively, and was 6-fold higher for total phenolic and total flavonoid content, and 10-fold higher for total flavonol content than in the mead without juice addition (CW) due to highest

Table 4 Composition of obtained meads

Parameter	Sample			
	CW	B5W	B10W	B20W
Dry matter content, %	4.88 ± 0.03^a	3.85 ± 0.00^b	3.85 ± 0.05^b	4.05 ± 0.05^c
pH	3.30 ± 0.06^a	3.10 ± 0.02^b	3.08 ± 0.02^b	3.07 ± 0.06^b
Acidity, g·L^{-1}	3.84 ± 0.03^a	4.59 ± 0.03^b	5.00 ± 0.02^c	5.39 ± 0.03^d
Volatile acidity, mg·L^{-1}	660.00 ± 60.00^a	560.00 ± 34.64^b	440.00 ± 34.64^c	400.00 ± 17.42^c
Ethanol content, % vol.	6.93 ± 0.03^a	7.55 ± 0.03^b	7.98 ± 0.08^c	7.84 ± 0.02^d
Reducing sugar content, g·L^{-1}	26.10	7.83	3.30	3.11

^{a,b,c,d,e,f} = significantly different ($P \leq 0.05$)

Table 5 Results of total polyphenols content and antioxidative activity of meads

	Total phenolic content, μgGAE·mL^{-1}	Total flavonol content, μgQE·mL^{-1}	Total flavonoid content,μgQE·mL^{-1}	ABTS, μgTE·mL^{-1}	DPPH, μgTE·mL^{-1}
Blackberry juice (raw)	498.71 ± 31.74	243.49 ± 4.72	209.11 ± 0.77	8.89±0.04	2.50±0.02
CW	79.83^{a} ± 10.16	6.06^{a} ± 0.21	8.87^{a} ± 0.30	0.40^{a} ± 0.06	2.29^{a} ± 0.15
B5W	160.56^{b} ± 4.36	22.15^{b} ± 1.09	21.74^{b} ± 0.35	0.63^{b} ± 0.00	6.89^{b} ± 0.03
B10W	261.68^{c} ± 5.33	34.77^{c} ± 0.29	29.66^{c} ± 2.26	0.65^{b} ± 0.01	6.98^{b} ± 0.03
B20W	490.88^{d} ± 13.35	62.57^{d} ± 0.57	50.34^{d} ± 1.11	0.54^{c} ± 0.00	5.18^{c} ± 0.17

^{a,b,c,d,e,f} = significantly different ($P \leq 0.05$)

amount of added blackberry juice. Altough the highest content of phenolic compounds was measured in B20W, the strongest antioxidant activity was determined in the B10W mead; it accounted for 6.98 μgTE·mL^{-1} (DPPH assay) and for 0.65 μgTE·mL^{-1} when analyzed with the ABTS assay, what was 1.5-fold and 3-fold higher, respectively, than in the mead without juice addition (CW). All honeys are rich sources of secondary metabolites with antioxidant activity, especially polyphenols. The main polyphenols are the flavonoids, and during fermentation process they are modified through polymerization and complexation with proteins [25]. This might be the answer why B20W had higher content of polyphenols but lower antioxidant activity than B5W and B10W. The differences in the antioxidant activity of meads assayed with DPPH and ABTS tests may result from differences in the kinetics of these tests and in the concentration of substrates (e.g. ABTS method is used for lipophilic and hydrophobic antioxidants, contrary to DPPH method) [23]. The literature provides data on the total concentration of polyphenols and antioxidant properties measured with the DPPH and ABTS assays in different types of meads. Socha *et al.* determined the highest concentration of total polyphenols in the mead with juice from rowanberry what was 45-fold lower compared to that measured in the mead with juice from red fruits of Cornelian cherry [3, 7]. In other studies on the antioxidant properties of mead, much lower antioxidant activity was obtained in meads obtained from different kinds of honey and in meads that differ in their production technology [9].

CONCLUSION

The conducted study demonstrated that the use of blackberry juice influenced the course of fermentation of meads as well as their physicochemical and antioxidative properties (positive effect on fermentation dynamics – almost 25% higher rate of fermentation than in control samples, and improvement of all physicochemical characteristics and composition of resultant meads). The strongest antioxidative activity and the most beneficial chemical composition were determined in the B10W mead. The graphs in Fig. 1. showed quite well overlapping of fitted curves with experimentally determined data.

CONTRIBUTION

A. Savić, A. Velemir, S. Papuga, M. Stojković conceived and designed the experiments; performed the experiments; analyzed the data; contributed reagents, materials and analytical tools; wrote the paper.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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