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SCIENTIFIC PAPER

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EFFECTS OF ADDING DIFFERENT QUANTITIES OF YEAST AND CHOKEBERRY JUICE ON FERMENTATION OF MEAD

Article Highlights

- Adding chokeberry juice improved the antioxidant properties of the final product
- Adding chokeberry juice positively affected the course of the fermentation of mead
- A statistically significant difference between the samples containing different quantities of yeast
- The model foresaw a fairly good overlap of curves with the experimentally obtained data
- The addition of aronia juice affects the antimicrobial activity of meads

Abstract

Honey is a product of high nutritional value, used as a raw material for obtaining mead. However, adding fruit juices, including chokeberry juice, can improve mead quality. This paper aims to assess the effects that adding different quantities of chokeberry juice, with the variation of 3 amounts of inoculated yeast, has on the fermentation and physicochemical, antioxidant, and antimicrobial properties of mead. The parameters analyzed are the dry matter content, pH value, and content of volatile acids, ethanol and methanol, total phenols and flavonoids, FRAP, DPPH, and ABTS tests, and antimicrobial properties. The results obtained in this paper show that adding chokeberry juice improves the antioxidant properties of the final product and positively affects the course of mead fermentation, i.e., it has led to an increase in the maximum concentration of ethanol. Regarding the chemical composition of mead, there is no significant difference, except in the obtained ethanol content, which is the highest in samples with 10% of added chokeberry juice. Furthermore, the control sample showed the best antimicrobial activity, while the sample with 5% added chokeberry juice showed the weakest effect. Finally, the strongest effect was seen in the sample with 20% of added chokeberry juice.

Keywords: antimicrobial activity, antioxidant activity, aronia, fermentation rate, kinetic model, mead.

Honeydew honey comes mainly from the excretions of plant-sucking insects (Hemiptera) on the living parts of plants or their secretions [1]. A particular characteristic of this type of honey is its high antioxidant [2] and antimicrobial activity [3].

Mead is a traditional alcoholic beverage that contains between 8 and 18 vol.% produced by the alcoholic fermentation of diluted honey under the influence of yeast cells [4]. Mead positively affects human metabolism, especially digestion, and reduces the risk of chronic diseases [5].

Numerous studies focus not only on traditional mead but also on mead with the addition of fruit juices, fruits, herbs, and spices [6]. Melomel is a special type of mead. It is obtained by adding fruits or juices to a honey solution [7]. These additives accelerate and ameliorate the fermentation process, increase alcohol yield, and improve the characteristics of the final product [8,9]. Due to the high nutritional value of berries and their antioxidant properties and specific taste, chokeberry (*Chokeberry melanocarpa*) is a fruit raw

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material used in the food industry more and more often [10]. Adding chokeberry to a honey solution might affect the antioxidant and antimicrobial properties of mead because its berries are a rich source of polyphenolic compounds and other bioactive components (proanthocyanidins, anthocyanins, flavonoids, phenolic acids, etc.), and the antimicrobial effect of chokeberry is demonstrated on a wide spectrum of microorganisms [11]. Besides, these components also have anti-inflammatory and antiviral activity [12].

Several mathematical models describing the process kinetics were used to achieve better control of the fermentation process. Generally, kinetic models of alcoholic fermentation provide a mathematical description of the processing speed under different conditions of temperature, pH value, aeration, mixing, parameters correction, regulation of the level of foam, etc. It enables the reduction of production costs and increases the quality of the final product. The Gompertz mathematical model can be applied to predict different substrates' fermentation kinetics [13].

This paper aims to examine the effect of adding different quantities of chokeberry juice in fermentation solutions, with a variation of 3 quantities of inoculated yeast, on the fermentation and physiochemical, antioxidant, and antimicrobial properties of mead.

EXPERIMENTAL

For the needs of this experiment, honeydew honey produced in 2018 in the Banjaluka region, Republic of Srpska, was used, as well as cold-pressed chokeberry juice produced by "Natural Agro" from Prnjavor, Republic of Srpska. In this experiment, the sample of the honeydew honey was kept in glass containers at 4 °C, and the chokeberry juice in plastic bottles at -18 °C.

Honeydew honey must preparation

Honeydew honey was stirred with water in a ratio of 1:3 (honeydew honey/water). The resultant wort was pasteurized at 65 °C for 10 min, cooled, and poured into fermentation flasks. Aronia juice was also pasteurized at 65 °C for 10 min, cooled, and poured into fermentation flasks in amounts required for this study. Four samples were prepared: control wort without added Aronia juice (sample 1) and three worts with added Aronia juice in the amount of 5% (sample 2), 10% (sample 3), and 20% (sample 4) of fermentation wort volume. Into all samples, yeast energizer (VitaFerm Ultra F3, Erbslöh, Geisenhein, Germany) was added in an amount of 0.267 g/L. Commercial yeast Fermol Lager (AEB Group, Italy), a selected dry yeast strain of *Saccharomyces pastorianus*, was rehydrated in distilled water at 35 °C–40 °C for 30 min and added in the amount of 0.15 (label A), 0.30 (label B) and 0.6 (label C) g/L of wort. The process of alcoholic fermentation was conducted at 25 °C for 21 days. All fermentations were carried out in triplicate using a system consisting of 250 mL flasks containing 190 mL of wort mixture and fitted with an airlock to release CO_2 produced during fermentation. The dynamics of the fermentation processes were controlled based on weighing the flasks in time on a scale every 24 h throughout the alcoholic fermentation [14].

General oenological parameters

At the end of fermentations, the oenological parameters of mead: pH value, volatile acidity, and dry matter content, were measured [15].

The content of ethanol and methanol in mead was determined by the GC-FID method at Clarus 680 Perkin Elmer instrument with the FID detector, Elite-Wax L 60 m column, ID 0.32, DF 0.5, absolute ethanol and methanol standards, with acetonitrile as the internal standard. The injector and detector temperature of 250 °C, a sample volume of 0.5 µL, and a temperature regime of 45 °C (2 min), 45 °C/min to 245 °C (1 min). The total duration was 7.44 min, and the flow was 3 mL/min [16].

Determination of antioxidant activity

The total phenolic content in meads was measured spectrophotometrically according to the Folin-Ciocalteu method [17]. The results were expressed as total phenolic equivalent to gallic acid (mg GAE/mL).

The total flavonoid content in meads was measured using the method of Ordoñez *et al.* [18]. The results were expressed as flavonoid content to gallic acid (mg GAE/mL). 2,2-Diphenyl-1-picrylhidrazyl (DPPH) radical scavenging assay was determined by the method of Brand-Williams *et al.* [19]. The results were expressed in µgTrolox equivalent/mL-(µg TE/mL). 2,2′-Azino-bis-(3 - ethylbenzothiazoline - 6- sulphonic acid) (ABTS) radical scavenging assay was performed [20]. The results were expressed in mg TE/mL. Finally, ferric reducing/antioxidant power (FRAP) was performed as described by Banzie and Strain [21]. The results were expressed in mmol Fe^{2+}/mL.

Antibacterial activity

The following bacterial cultures: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 10145, *Staphylococcus aureus* ATCC 25923, and *Bacillus cereus* ATCC 7004 were used. The cultures were grown in a Nutrient broth (Liofilchem, Italy) and incubated for 24 h at 37 °C, after which they were inoculated and grown on Nutrient agar (Liofilchem,Italy)

for the next 24 h at 37 °C. Agar wells and agar dilution methods for determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) [22,23]. The concentration of the mead in the medium was 40%, 30%, and 20% (v/v). The highest dilution of the tested mead to inhibit the visible growth of bacteria was considered the MIC value. From the plates showing no visible sign of growth in MIC determination, test microorganisms were inoculated onto sterile Mueller Hinton agar (Liofilchem, Italy) plates. The plates were then incubated at 37 °C for 24 h. The lowest concentration that did not show test organism growth was considered the MBC. The antimicrobial activity of meads was performed in eight replicates for the agar wells method and two replicates for the MIC/MBC method. The results were expressed as the mean ± SD for the agar wells method and in % (v/v) of mead. The antibiotic susceptibility discs Ampicillin (10 μg), Gentamicin (10 μg), Erythromycin (15 μg), and Ciprofloxacin (5 μg) were used as a positive control. The manufacturer of antibiotic discs is Mast Group, UK.

Statistical analysis

All tests were performed in two or three replicates (except the agar wells method), and the results were expressed as means ± standard deviation (except for MIC/MBC values). Analysis of variance (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 by applying linear regression analysis and Fisher's statistical test.

RESULTS AND DISCUSSION

The fermentation process kinetics was monitored based on measuring the changes in the mass of the bottles in certain time intervals, and it was expressed as a cumulative mass (g) of the ethanol produced in a specific time interval. The production of CO_2 during alcoholic fermentation represents an indirect measure of the consumption of fermentable carbohydrates [14]. Figures 1, 2, and 3 show the kinetics of ethanol production in the fermentation process for 12 samples. A great similarity can be noticed between the curves representing the fitted curves determined by a model and the curves determined experimentally. The correlation coefficient ranges from 0.984 to 0.999. Furthermore, an exponential increase in the number of yeast cells can be seen in all the samples.

Table 1. Kinetic parameters of the developed mathematical models and corresponding experimental data.

Sample	P_m, g,cumulative mass	$r_{p,m}$, g·h^{-1}	t_1, h	R^2
1A	152.39	0.24	192.44	0.990
	74.31*	0.02*	-	
1B	96.48	0.32	51.66	0.998
	92.40*	0.06*	-	
1C	93.54	0.26	35.34	0.997
	86.33*	0.06*	-	
2A	111.57	0.37	46.18	0.987
	113.47*	0.09*	-	
2B	98.77	0.35	43.95	0.983
	102.44*	0.05*	-	
2C	99.66	0.42	37.52	0.985
	105.12*	0.08*	-	
3A	100.54	0.42	43.20	0.996
	102.41*	0.08*	-	
3B	101.36	0.43	37.09	0.997
	103.29*	0.13*	-	
3C	101.76	0.42	25.22	0.995
	104.45*	0.10*	-	
4A	98.58	0.35	45.79	0.990
	101.39*	0.10*	-	
4B	99.75	0.37	40.31	0.991
	102.70*	0.12*	-	
4C	94.80	0.36	33.43	0.989
	98.56*	0.14*	.	

* - measured values.

Table 1 shows in a parallel manner the values of kinetics parameters of the developed equations and corresponding values calculated based on the experimental data. For all the samples, the predicted values correspond to the measured ones, except for sample 1A. The reason for this deviation could be the lowest amount of yeast added compared to all three control samples. In most samples, the maximum ethanol production rate (R_{pm}) calculated from the experimental data was higher than the predicted values.

The lowest P_m-value (74.31) was obtained from sample 1A, while the highest value (113.47) was obtained from sample 2A. The values of r_{pm} ranged from 0.02. Based on the results shown in Table 1 and Figures 1, 2, and 3, there is a statistically significant difference between the samples containing the same amount of added chokeberry juice, *i.e.*, between

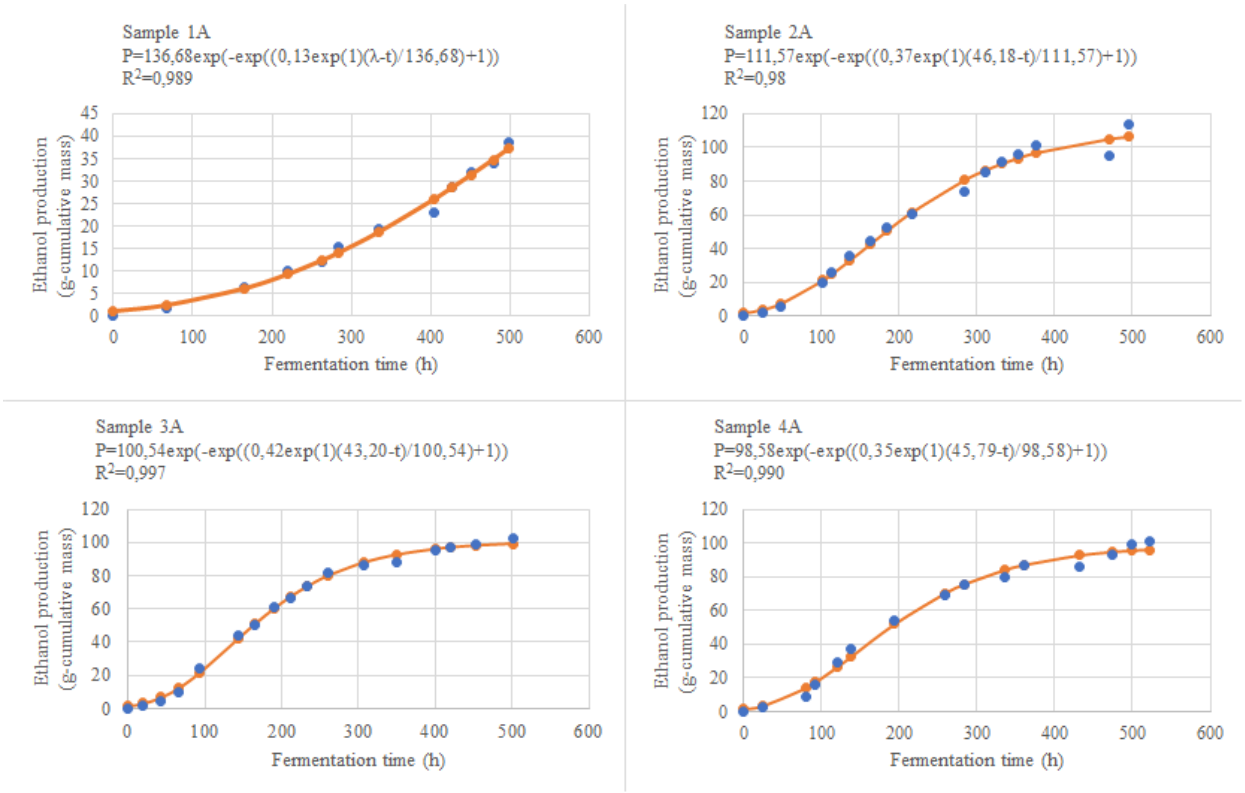

Figure 1. Ethanol production kinetic and results from fitting the experimental data into a modified Gompertz equation (solid line - fitted curve, symbol - experimental data) for samples 1A, 2A, 3A and 4A.

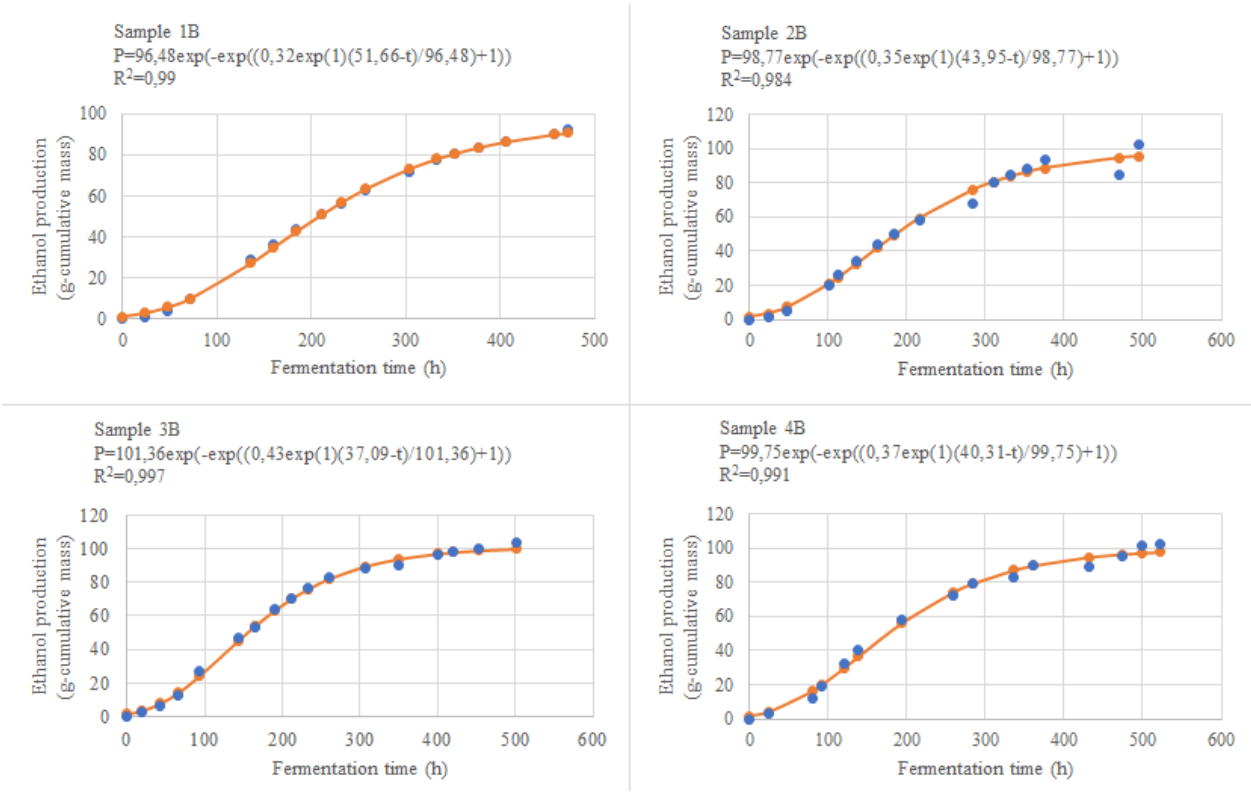

Figure 2. Ethanol production kinetic and results from fitting the experimental data into a modified Gompertz equation (solid line - fitted curve, symbol - experimental data) for samples 1B, 2B, 3B, and 4B

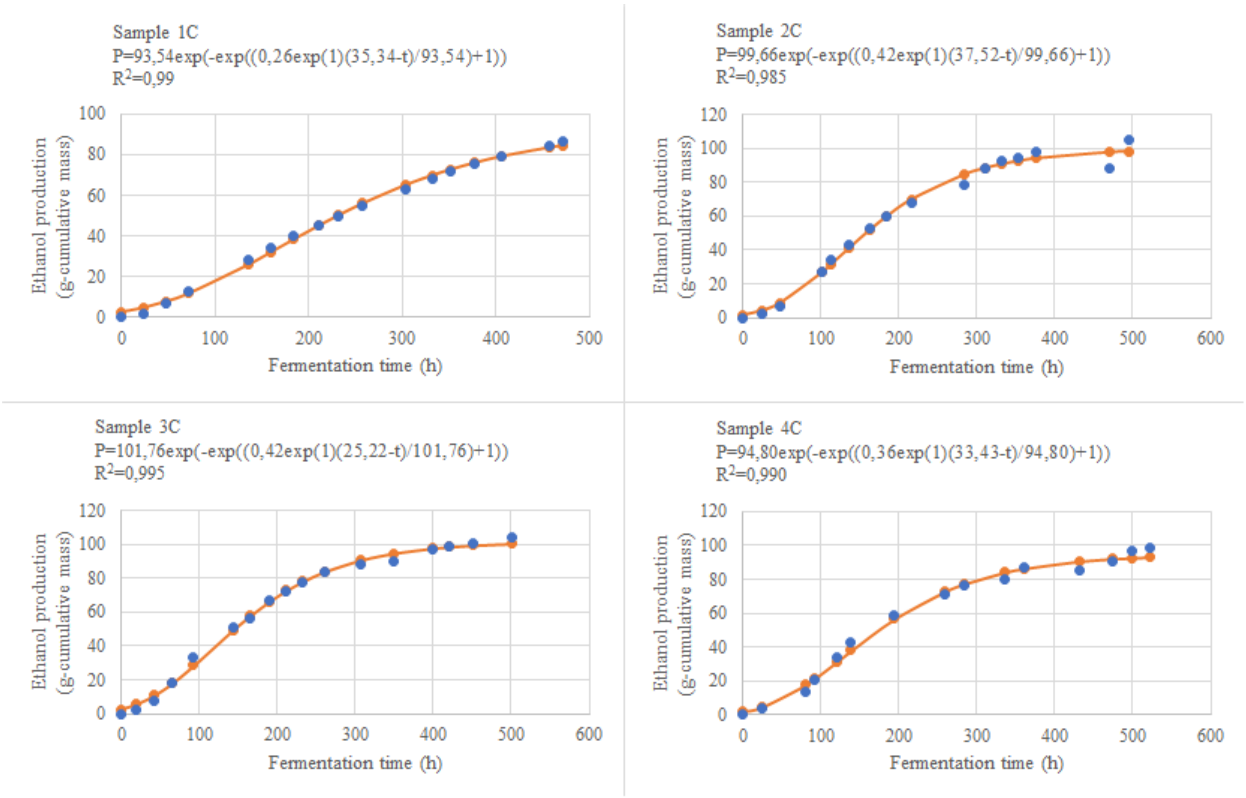

Figure 3. Ethanol production kinetic and results from fitting the experimental data into a modified Gompertz equation (solid line - fitted curve, symbol - experimental data) for samples 1C, 2C, 3C and 4C

different quantities of yeast added to start the fermentation process. for sample 1A, to 0.14 for sample 4C. In addition to these values, a significant difference was noticed in the duration of the lag phase, which was the shortest in sample 3C (25.22 h), while the longest lag phase was measured in sample 1A (192.44 h).

Table 2 shows the results of the dry matter content and pH in stock solutions for fermentation. It can be noticed that the addition of chokeberry juice led to a mild decrease in the dry matter content, from 18.50% in the control sample to 18.00% in the sample with the addition of 20% of chokeberry juice. The fermentation process led to a reduction of the dry matter content, which is shown in Table 3, and once the fermentation was over, it ranged from 7.93% for sample 1B to 9.48% for sample 2B. The statistical analysis showed that in one part of the mead samples, there was no statistically significant difference in the results of the dry matter content with the same quantity of added chokeberry juice. In contrast, for samples 2 and 4, a significant statistical difference was noticed. Based on the obtained results, it can be presumed that mead contains a higher amount of residual sugar that does not participate in the fermentation process [24], *i.e.*, that such dry matter content can be explained by the presence of sugar in the form of disaccharides and oligosaccharides, which cannot be fermented by yeast [25].

Table 2. The results of physicochemical parameters of honey must (pH value and dry matter content).

Parameter/sample	1	2	3	4
Dry matter content (%)	18,50 ± 0,00	18,50 ± 0,00	18,30 ± 0,00	18,00 ± 0,00
pH	3,99 ± 0,00	3,95 ± 0,01	3,91 ± 0,01	3,85 ± 0,01

The pH value of the must prepared by mixing honeydew honey and water was 3.99. By adding chokeberry juice, the pH in all musts decreased. By adding 5%, 10%, and 20% of juice, the pH dropped to 3.95, 3.91, and 3.85, respectively. The decrease in pH could be due to the naturally high acidity of chokeberry juice. Once the fermentation process was over, pH dropped in all samples. The lowest pH of 3.54 was in sample 1B, while the highest value of 3.80 was in sample 4C. Sroka and Satora [26] obtained similar results of pH, where the mead pH was 3.40, while Martínez *et al.* [27] stated that pH ranged from 3.66 to 4.00. In most of the mead samples with the same juice content, there was no significant statistical difference in

pH, indicating that the quantity of yeast does not significantly impact the change of pH in the fermentation process. The trend of changes in pH depends on the acidity derived from fruit juice. In contrast, the decrease in pH during fermentation can be a consequence of the metabolic activity of yeast [28]. A pH decrease after fermentation can also be explained by the weak buffering capacity of honey [29] and by the production of acids by yeast during fermentation [30]. Low pH in fermentation inhibits microbial growth, leading to product spoilage. At the same time, it creates a suitable environment for the growth of microorganisms necessary for the fermentation process [28].

Table 3 shows the results of the physicochemical analysis of mead. Volatile acids in honey are mostly a result of the production of acetic acid during yeast fermentation. This acid, in alcoholic fermentation, is produced by yeast *S. Cerevisiae* in the amount of 0.3 g/L to 0.8 g/L, even though the formation of this compound is undesirable [31]. The content of volatile acids is affected by the type of yeast used, fermentation conditions, and the chemical composition of raw material [32,33]. Based on the results shown in Table 3, it can be noticed that the content of volatile acids in mead significantly differs with the same amount of added chokeberry juice and different concentration of added yeast. Bely *et al.* [34] state that during the fermentation of solutions with higher amounts of sugar, the amount of yeast added did not significantly affect the content of volatile acids in the final product, which is different from the results obtained in this paper. The prolonged fermentation leads to an increase in the content of volatile acids and a decrease in pH [35]. The content of volatile acids in the mead ranged from 0.14 g/L in sample 4A to 0.33 g/L in sample 1C. The content of volatile acids should not be higher than 1.4 g/L [32], following the results obtained in this paper. The production of a larger amount of acetic and succinic acid can slow or even stop fermentation [8].

The ethanol content in the mead ranged from 8.32 vol.% in sample 4B to 11.98 vol.% in sample 3A.

Table 3. The results of physicochemical parameters od meads (pH value, dry matter content, volatile acidity, methanol content and ethanol content).

Sample	Dry matter content (%)	pH	Volatile acidity (g/L)	Methanol content (vol. %)	Ethanol content (vol. %)
1A	$8,10^{a}\pm0,09$	$3,61^{b}\pm0,02$	$0,27^{fg}\pm0,05$	$0,001^{a}\pm0,000$	$8,65^{a}\pm0,35$
1B	$7,93^{a}\pm0,31$	$3,54^{a}\pm0,01$	$0,29^{gh}\pm0,03$	$0,002^{ab}\pm0,001$	$9,19^{a}\pm0,28$
1C	$8,15^{a}\pm0,25$	$3,56^{a}\pm0,02$	$0,33^{h}\pm0,04$	$0,002^{a}\pm0,001$	$9,13^{a}\pm0,53$
2A	$9,33^{de}\pm0,05$	$3,69^{cd}\pm0,01$	$0,21^{cde}\pm0,03$	$0,002^{ab}\pm0,000$	$11,39^{bc}\pm0,14$
2B	$9,48^{e}\pm0,36$	$3,72^{d}\pm0,02$	$0,24^{ef}\pm0,00$	$0,001^{a}\pm0,001$	$11,15^{bc}\pm0,34$
2C	$9,02^{cd}\pm0,34$	$3,72^{d}\pm0,00$	$0,23^{def}\pm0,02$	$0,000^{a}\pm0,000$	$10,79^{b}\pm0,40$
3A	$8,58^{bc}\pm0,08$	$3,66^{c}\pm0,01$	$0,19^{bcd}\pm0,03$	$0,001^{a}\pm0,000$	$11,98^{c}\pm0,19$
3B	$8,62^{bc}\pm0,10$	$3,69^{cd}\pm0,01$	$0,18^{abc}\pm0,00$	$0,001^{a}\pm0,000$	$11,72^{bc}\pm0,31$
3C	$8,62^{bc}\pm0,12$	$3,73^{d}\pm0,01$	$0,21^{cde}\pm0,00$	$0,001^{a}\pm0,000$	$11,66^{bc}\pm0,16$
4A	$8,70^{abc}\pm0,28$	$3,77^{e}\pm0,03$	$0,14^{a}\pm0,02$	$0,004^{bc}\pm0,001$	$8,61^{a}\pm0,15$
4B	$8,60^{b}\pm0,00$	$3,78^{e}\pm0,00$	$0,15^{ab}\pm0,03$	$0,003^{ab}\pm0,002$	$8,33^{a}\pm0,39$
4C	$8,75^{bc}\pm0,08$	$3,80^{e}\pm0,00$	$0,16^{ab}\pm0,03$	$0,006^{c}\pm0,002$	$9,03^{a}\pm0,39$

Higher ethanol content was measured in the samples in which 5% and 10% of chokeberry juice had been added. The ethanol content was lower in the samples without and with 20% added chokeberry juice. Akalin *et al.* [33] presented similar results, recording the ethanol content from 9.20 vol.% to 11.38 vol.%, while Pereira *et al.* [36] recorded from 10.03 vol.% to 10.33 vol.%. The ethanol content in mead depends on the yeast used in the fermentation process and the additives used in its production [37]. Lower ethanol content and a shorter fermentation period can be explained by the poorer response of yeast cells to stress conditions in the fermentation solution [38]. Similar results can be found in the study of Martínez *et al.* [27]. Akalin *et al.* [33] stated that the alcohol content in mead mainly depended on the quantity of honey in the stock solution and its dilution level. Based on the results shown in Table 3, it can be seen that in most samples, there are no statistically important differences in the ethanol content for the mead in which the same amount of juice was added, *i.e.*, in the samples with different concentrations of added yeast. Roni *et al.* [39] stated that adding a higher yeast concentration in the bioethanol production resulted in a higher ethanol concentration, which differed from the results of the present work. Ethanol produced in the fermentation process helps preserve, extract, and absorb phenolic compounds naturally present in honey [40].

The methanol content in mead was very low. In sample 2C, the methanol content was lower than 0.001 vol.%, while in sample 4C, the measured content was 0.006 vol.%. The methanol content in the mead

derives from the added chokeberry juice because the content and esterification degree of pectic matters in fruit can affect the methanol concentration in wines, thus also in mead [41]. Based on the obtained results, it can be seen that there is no significant difference between individual samples and that the addition of chokeberry juice did not significantly affect the increase of methanol in mead.

Table 4 shows the results of the analysis of antioxidant properties, *i.e.*, the total phenolic and flavonoid content and the FRAP, DPPH, and ABTS tests. The total phenolic content in the analyzed samples ranged from 297.61 mg GAE/mL in sample 1C to 715.62 mg GAE/mL in sample 4B. Based on the results shown in Table 4, it can be seen that the increase of the added chokeberry juice led to an increase in the total phenolic content. A similar can be noticed with the flavonoid content, where the flavonoid content was lowest in the control samples (1A, 1B, 1C), in which chokeberry juice had not been added. In contrast, the highest flavonoid content was measured in those samples in which 20% of chokeberry juice had been added (4A, 4B, 4C). The statistical analysis shows that the total phenolic content was different in the same amount of added juice, *i.e.*, there were differences in the phenolic content when adding different yeast concentrations. Unlike the total phenolic content, the flavonoid content in the samples with the same amount of juice and different yeast concentration was not statistically different in most samples, except for the samples with 5% chokeberry juice added. Also, based on the results, it can be concluded that adding fruit or fruit juice to the production of mead leads to an increase in the total phenolic and flavonoid content and the overall antioxidant capacity [42]. Similar results were obtained by Adamenko *et al.* [37], who found that mead with the addition of dogwood juice had a significantly higher total phenolic content than the control samples. The total phenolic content in the samples with the added juice was 898.7 mg GAE/L, which is higher than the results obtained in this paper. Kawa-Rygielska *et al.* [32] analyzed mead by adding grape seeds. It was determined that adding this component to the mead increased the total phenolic content in the final products. The content of polyphenolic compounds affects the quality of food products, especially their color, aroma, bitterness, and antioxidant activity [32]. The phenolic content changes during the technological process of production, especially during fermentation, followed by temperature treatment and storage [43].

Table 4. Total phenolic and flavonoid content and antioxidant activity of meads (FRAP, DPPH and ABTS assays).

Sample	Total phenols (mg GAE/mL)	Total flavonoids (mg GAE/mL)	FRAP (mmol Fe^{2+}/mL)	DPPH (μg TE/mL)	ABTS (mg TE/mL)
1A	$312,75^{a}$±1,61	$177,13^{ab}$ ±4,46	$1,99^{a}$±0,01	$42,54^{a}$ ± 0,42	$11,78^{f}$ ± 0,42
1B	$322,28^{a}$ ± 1,80	$188,67^{ab}$ ± 13,70	$2,02^{a}$ ± 0,09	$39,52^{a}$ ± 0,71	$11,31^{ef}$ ± 0,31
1C	$297,61^{a}$ ± 3,39	$156,33^{a}$ ± 11,99	$1,97^{a}$ ± 0,00	$43,59^{a}$ ± 0,90	$10,40^{e}$ ± 0,16
2A	$391,21^{c}$ ± 6,18	$232,93^{c}$± 5,10	$3,36^{c}$ ± 0,01	$157,13^{b}$ ± 13,19	$6,74^{cd}$ ± 0,38
2B	$359,21^{b}$ ± 4,10	$194,67^{b}$± 4,94	$3,31^{bc}$ ± 0,02	$169,62^{bc}$ ± 11,05	$6,74^{cd}$ ± 0,30
2C	$371,61^{bc}$ ± 7,57	$200,93^{b}$± 5,99	$3,27^{b}$ ± 0,02	$191,16^{c}$ ± 15,04	$5,99^{bc}$ ± 1,30
3A	$466,68^{d}$ ± 3,94	$293,00^{d}$ ± 2,74	$4,48^{d}$ ± 0,06	$362,33^{d}$ ± 14,30	$6,97^{cd}$ ± 0,19
3B	$486,88^{de}$ ± 3,29	$308,13^{d}$ ± 4,30	$4,53^{d}$ ± 0,04	$390,01^{e}$ ± 12,67	$7,02^{d}$ ± 0,16
3C	$499,88^{e}$± 6,07	$316,93^{d}$ ± 1,42	$4,91^{e}$ ± 0,03	$387,37^{d}$ ± 17,38	$5,24^{b}$ ± 0,30
4A	$689,36^{f}$ ± 9,52	$474,88^{e}$ ± 11,05	$5,27^{f}$ ± 0,05	$577,42^{g}$ ± 15,46	$3,77^{a}$ ± 0,61
4B	$715,62^{g}$ ± 13,71	$490,61^{e}$ ± 13,12	$5,72^{g}$ ± 0,05	$513,25^{f}$ ± 21,65	$5,12^{b}$ ±0,23
4C	$705,76^{fg}$ ± 37,83	$471,61^{e}$ ± 37,91	$5,98^{h}$ ± 0,02	$559,23^{g}$ ± 21,88	$5,06^{b}$ ± 0,45

The results of the FRAP test ranged from 1.97 mmol Fe^{2+}/mL for sample 1C to 5.98 mmol Fe^{2+}/mL for sample 4C. The results in Table 4 indicate that by increasing the quantity of added chokeberry juice, the FRAP value also increases, *i.e.*, adding 5%, 10%, and 20% chokeberry juice increases the FRAP value by 1.6 times, 2.33 times, and 2.84 times, respectively. Adamenko *et al.* [37] state that the FRAP value in the samples with added dogwood juice was 8 to 39 times higher than in the control samples, which represents more significant changes compared to the changes achieved in this experiment, while Kawa-Rygielska *et al.* [32] state that the addition of chokeberry juice leads to twice as high FRAP values compared to the control samples, which is very similar to the results of this paper.

The result of the DPPH test was lowest in the control sample 1B (39.52 μg TE/mL), while the highest value was measured in sample 4A (577.42 μg TE/mL). Based on the results of the DPPH test, it can be noticed that the increased amount of the added chokeberry juice leads to an increase in the DPPH value. Kawa-Rygielska *et al.* [32] stated that by adding chokeberry

juice to the fermentation solution, the DPPH value increased significantly, up to 3 times higher than the control sample, which was similar to samples 2 to which 5% of chokeberry juice was added; with 20% of the juice added, the DPPH value increased by about 13 times.

The ABTS test involved the IC50 method, where higher values indicated weaker antioxidant properties of the sample. The highest ABTS values were measured in sample 1A (11.78 mg TE/mL), while the lowest values were measured in sample 4A, 3.77 mg TE/mL. The highest ABTS values were recorded in the control samples, while the lowest values were found in the samples with the highest amount of chokeberry juice added. The total phenolic and flavonoid content, FRAP, DPPH, and ABTS tests showed that adding chokeberry juice improved antioxidant properties. The antioxidant activity of mead depends on the chemical composition of raw material but also on the production technology, chemical composition, and additives (fruits, herbs, and spices), which confirms that the addition of chokeberry juice can affect the total antioxidant capacity of a final product [32]. Adamenko *et al.* [37] showed that mead with added dogwood juice had stronger antioxidant properties measured by the DPPH and ABTS tests than the control mead. Also, the results of the FRAP, DPPH, and ABTS tests were mainly significantly different for samples with the same amount of added juice and different concentrations of yeast. The impact of the yeast concentration on the antioxidant capacities of mead, but also other alcoholic beverages, still has not been researched sufficiently, nor can more significant literature be found, which could represent an idea for future research.

Table 5 shows the results of testing the antibacterial activity of mead by applying the agar-well method and agar dilution method for determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) [22,23]. The results obtained using the agar-well method showed no inhibitory effect against the tested bacteria. The disadvantage of the agar-well method is the relatively long incubation period necessary to detect an inhibition zone that might lead to the evaporation of volatile or the degradation of thermally unstable agents, as well as a small amount of mead used for testing (30 µL), which probably caused the absence of the antibacterial effects of the tested mead. Also, it is impossible to quantify the amount of the antimicrobial agent diffused into the agar medium due to the gradient and matrix network of the agar used for the assay [22]. Because the agar is an aqueous preparation, non-polar compounds will not diffuse as well as polar compounds. Eloff *et al.* [44] have shown that the intermediate polarity compounds have the highest antimicrobial activity. The agar-diffusion method could be useful with a single compound with a known polarity. Even in such a case, if the positive control's polarity differs much from the single compound, comparisons may not be valid [45].

On the other hand, MIC and MBC values showed accurate, reproducible, and reliable results. The MIC and MBS values of mead concerning four bacteria are shown in Table 5. In most cases, sample 1 (the control sample) showed the best antimicrobial activity, while sample 2 (with 5% added chokeberry juice) showed the weakest effect. Following the control sample, the sample with 20% of added chokeberry juice showed the strongest effect, which still indicates the impact the quantity of added juice has on the antimicrobial effect of mead. The obtained results are not in compliance with the results of measuring the antimicrobial effect, where it has been found that adding chokeberry juice positively affected the antioxidant properties of mead. In the available literature, there is not much data on the antimicrobial effect of mead. Still, data on the antimicrobial effect of similar products, such as beer, wine, and related products, are available. The influence of different factors has been tested with these products, such as the type and quantity of raw materials used, amount of alcohol, acidity, the content of phenolic compounds, etc. A lot of data on the antimicrobial effect of honey and chokeberry, the raw material used in this paper, can be found in the literature. Stojković *et al.* [46] tested the antibacterial effect of honeydew honey, processed through various treatments, on four bacteria also used in this paper. This study showed that G (-) bacteria were more strongly inhibited than G (+) bacteria, which is not in line with the results from many authors [47,48]. The results of this study are interesting because of the phenolic content of sample 4, which showed that the strongest antibacterial effect was among the lowest measured, similar to mead without added chokeberry juice. Hence, it follows that the antimicrobial properties of honeydew honey could be attributed to the individual or synergetic effects of different factors, not only from phenolic compounds' content. The antimicrobial activity of honey is affected by a number of factors, such as high osmotic pressure, water activity, pH value, production of H_2O_2, methylglyoxal, antimicrobial peptide bee defensin-1, lysozyme, phenolic acids, flavonoids, etc. [47,49]. The peroxide activity of honey may be destroyed by heat, light, and long-term storage of honey, and the antimicrobial activity of honeydew honey and honeydew mead could therefore be explained by the combined effect of the non-peroxide (high concentration of phenolics and flavonoids) and the

Table 5. Antimicrobial activity of meads and antibiotic discs (diameter of inhibition zone), minimum inhibitory (MIC), and minimum bactericidal concentration (MBC) of meads on bacterial cultures growth

	Sample	*Escherichia coli ATCC 25922*	*Staphylococcus aureus ATCC 25923*	*Pseudomonas aeruginosa ATCC 10145*	*Bacillus cereus ATCC 7004*
1A	Disc-diffusion (mm)	*NA	*NA	*NA	*NA
	MIC % (v/v)	30	30	30	30
	MBC % (v/v)	40	40	40	40
1B	Disc-diffusion (mm)	*NA	*NA	*NA	*NA
	MIC % (v/v)	30	30	30	30
	MBC % (v/v)	40	40	40	40
1C	Disc-diffusion (mm)	*NA	*NA	*NA	*NA
	MIC % (v/v)	30	30	30	30
	MBC % (v/v)	40	40	40	40
2A	Disc-diffusion (mm)	*NA	*NA	*NA	*NA
	MIC % (v/v)	40 %	>40 %	40 %	40 %
	MBC % (v/v)	>40 %	>40 %	40 %	>40 %
2B	Disc-diffusion (mm)	*NA	*NA	*NA	*NA
	MIC % (v/v)	40 %	>40 %	40 %	40 %
	MBC % (v/v)	>40 %	>40 %	40 %	>40 %
2C	Disc-diffusion (mm)	*NA	*NA	*NA	*NA
	MIC % (v/v)	40 %	>40 %	40 %	40 %
	MBC % (v/v)	>40 %	>40 %	40 %	>40 %
3A	Disc-diffusion (mm)	*NA	*NA	*NA	*NA
	MIC % (v/v)	40	40	30	30
	MBC % (v/v)	40	>40	40	>40
3B	Disc-diffusion (mm)	*NA	*NA	*NA	*NA
	MIC % (v/v)	40	40	30	30
	MBC % (v/v)	40	>40	40	>40
3C	Disc-diffusion (mm)	*NA	*NA	*NA	*NA
	MIC % (v/v)	40	40	30	30
	MBC % (v/v)	40	>40	40	>40
4A	Disc-diffusion(mm)	*NA	*NA	*NA	*NA
	MIC % (v/v)	30	30	30	30
	MBC % (v/v)	40	>40	30	>40
4B	Disc-diffusion(mm)	*NA	*NA	*NA	*NA
	MIC % (v/v)	30	30	30	30
	MBC % (v/v)	40	>40	30	>40
4C	Disc-diffusion(mm)	*NA	*NA	*NA	*NA
	MIC % (v/v)	30	30	30	30
	MBC % (v/v)	40	>40	30	>40
Ampicillin 10 mg (mm)		16.00±4.39	33.00±2.16	*NA	10.88±2.53
Ciprofloxacin 5 mg (mm)		37.00±3.37	29.25±2.99	33.33±0.58	27.38±3.54
Erytromycin 15 mg (mm)		10.75±1.50	27.75±2.99	*NA	24.5±2.52
Gentamicin 10 mg (mm)		25.50±1.29	29.75±1.90	21.33±1.53	24±2.53

*NA- no activity.

peroxide antimicrobial activity, low pH value, high concentration of sugar, etc. Fikselova *et al.* [50] studied the antimicrobial activity of several kinds of honeydew honey and found that the most sensitive bacteria strains were *E. coli* and *P. aeruginosa*, while *B. cereus* was the most resistant. In contrast, Srećković *et al.* [51] found

that honeydew honey was more effective against G (+) bacteria compared to G (-). Nicodim *et al.* [52] examined the effect of temperature on the antibacterial activity of honey and found that the lower temperatures (30 °C and 50 °C) did not have any effect on its antibacterial properties, while the antibacterial activity was reduced at the temperature of 70 °C.

The content of alcohol in mead, which was more or less balanced, did not affect the antibacterial properties of mead, which is in accordance with the previous studies related to wine, in which the content of alcohol ranged from 10% v/v to 13% v/v, where it was too low to show the bactericidal effect [53,54]. Radovanović *et al.* [54] and Vulić *et al.* [55] determined that the combination of organic acids (lactic, malic, acidic, and tartaric) and ethanol contributed to this stronger antimicrobial effect of the wine. However, the role of phenolic compounds is not completely clear because different opinions can be found in the literature. In their studies, Sheth *et al.* [56] and Arima *et al.* [57] suggested that compounds such as flavonoids (quercetin and quercetin-3-glucoside) and monomeric anthocyanins might be used as biochemical markers that contributed to the antimicrobial activity of red wines. However, Boban *et al.* [58], to clarify the role of polyphenols, pH, ethanol, and other wine components, tested the antimicrobial effects of intact wine compared to that of phenols-stripped wine, dealcoholized wine, ethanol, and low pH applied separately and in combination. They concluded that the antibacterial activity of the samples could not be related to their total phenolics and resveratrol content, ethanol content, or pH. After intact wine, the phenols-stripped wine had the strongest antimicrobial effect against *Salmonella enterica* and *E. coli*, so the authors concluded that nonphenolic constituents of wine were responsible for a major part of its antimicrobial activity. Krisch *et al.* [59] tested the antibacterial effect of extracts and juices of many different fruits. They determined that juices and extracts had low pH (from 2.8 to 5.5), which originated from weak organic and phenolic acids, which in the undissociated form (mainly on pH 3–5) can interact with cell membranes and penetrate the cells causing acidification of the cytoplasm. However, in their experiment, there was only a weak correlation between the acidity of the samples and their antibacterial effect. Krstić [60] tested the alcohol extract and chokeberry juice and determined the absence of the effect of juice on a large number of bacteria tested through the agar-well method. In contrast, MBC values in the juice were mainly over 20 mg/mL.

When preparing the mead, honey was diluted in a 1:3 ratio, which reduced the initial concentration of honey in a sample. Then, it was pasteurized, which might have influenced the antibacterial properties of the mead. Besides, the juice was pasteurized too, which also might have influenced the antibacterial properties of mead, even though the antioxidant properties were preserved. Further studies are needed to clarify the mechanism of the antimicrobial action of different compounds of meads. Still, the antimicrobial activity of a complex solution such as mead is based on more than one compound.

CONCLUSION

Adding chokeberry juice improved the final product's antioxidant properties and positively affected the course of the fermentation of mead with a fairly good overlap of the curves predicted by the model with the experimentally obtained data. Regarding the chemical composition of the mead, there is no significant difference, except in the content of obtained ethanol, which is the highest in the samples with 10% of added chokeberry juice. The control sample showed the best antimicrobial activity, while the sample with the least amount of added chokeberry juice showed the weakest effect. Among the samples with added chokeberry juice, the sample with the highest amount of added juice showed the strongest effect, which still indicated the impact of the quantity of added juice on the antimicrobial effect of mead.

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NAUČNI RAD

UTICAJ DODATKA RAZLIČITIH KOLIČINA KVASCA I VOĆNOG SOKA OD ARONIJE NA FERMENTACIJU MEDOVINE

Med predstavlja nutritivno visoko vrijedan proizvod, koji se kao sirovina koristi za dobijanje medovine. Kvalitet medovine može da se poboljša dodatkom voćnih sokova, a među njima i sokom od aronije. Cilj ovog rada je ispitivanje uticaja dodatka različitih količina soka od aronije (5%, 10% i 20%) u rastvore za fermentaciju, uz varijaciju 3 količine inokuliranog kvasca (150 mg/l, 300 mg/l i 600 mg/l), na tok fermentacije, fizičko-hemijska, antioksidativna i antimikrobna svojstva medovina. Od fizičko-hemijskih parametara u medovini je analiziran sadržaj suve materije, pH vrednost i sadržaj isparljivih kiselina, etanola i metanola. Analiza antioksidativnih svojstava je podrazumevala je određivanje sadržaja ukupnih fenola i flavonoida, FRAP, DPPH i ABTS testove. Testiranje antimikrobnih svojstava medovina vršeno je primenom dve metode. Rezultati dobijeni u ovom radu ukazuju da je dodatak soka od aronije poboljšao antioksidativna svojstva finalnog proizvoda, a pozitivno je uticao i na tok fermentacije medovine, odnosno doveo je do povećanja maksimalne koncentracije etanola (Pm). U pogledu hemijskog sastava medovina ne postoji značajna razlika, osim u sadržaju dobijenog etanola, koji je najveći kod uzoraka sa 10% dodanog soka od aronije. Uzorak 1 (kontrolni uzorak) pokazao je najbolju antimikrobnu aktivnost, dok je najslabije dejstvo pokazao uzorak 2 (sa 5% dodanog soka od aronije). Nakon kontrolnog uzorka, najjače dejstvo pokazao je uzorak sa 20% dodanog soka od aronije.

Ključne reči: antimikrobna aktivnost, antioksidativna aktivnost, aronija, brzina fementacije, kinetički model, medovina.