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Development and shelf-life assessment of soft-drink with honey

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ABSTRACT
This scientific work describes research that aims to determine the physicochemical parameters of homogenized honey and its safety indicators based on the determination of toxic metals and radionuclides. A series of experimental studies were conducted to develop and study recipes for honey water based on different types of honey collected in the Lviv region of Ukraine, namely acacia, buckwheat, sunflower, coriander, goldenrod, linden, and weeds. According to the results of experiments, it was found that the studied honey meets all the requirements presented in the standard for natural honey. And the results obtained to determine the dry matter content and pH allowed to blend different types of honey and get honey drinks, which will expand the range of non-carbonated products, which is very popular, especially in summer, and drink this drink during the year. To prolong the shelf life of honey drinks, it is recommended to add citric acid in an amount of 1% by weight of the drink and sodium benzoate as a preservative in an amount of 0.1%. The quality of the obtained honey water samples was assessed using organoleptic evaluation and physicochemical parameters. The resulting beverages have good organoleptic characteristics and can be offered for products in the industry.

Keywords: honey, water, beverage, dry matter content, pH value, formulation

INTRODUCTION
Beverage preparation is a component of food culture. And this is an integral part of national culture in general. Ukrainian drink "drinking honey" has a rich history, based on the knowledge of many generations of our ancestors **[1]**. Such beverages belong to the products of alcoholic fermentation of aqueous solutions of natural honey **[2]** and contain biologically active substances of honey and the use of only natural raw materials **[3]**. The need to form a culture of beverage consumption creates a search for the production of natural and environmentally friendly raw materials (fruit and berry, honey). This allows you to expand the range of buyers and the range of beverages made from natural raw materials **[4]**, **[5]**. Both ancient recipes for fermented honey drinks and drinks created thanks to modern scientific achievements are known **[6]**, **[7]**. The production of beverages based on natural raw materials should solve the interconnection and balance of ingredients, which form the taste and aromatic basis of the drink **[8]**. Usually, soft drinks consist of water, sweetener, and flavouring. Sugar sweeteners are sugar, glucose-fructose syrup, or other sweeteners (in the case of diet drinks). Drinks may also contain caffeine other components **[9]**. Use in the production of soft drinks based on honey can be promising. According to the Food and Agriculture Organization of the United Nations (FAO), honey exports from Ukraine for 11 months of 2020 reached an absolute record – 69.8 thousand tons with a total value of $ 117.5 million. In Figure 1, you can see how rapidly honey exports from Ukraine over the past 9 years **[10]**.

The previous record was set in 2017 and amounted to – 67.8 thousand tons ($ 133.9 million). Volumes of honey production in Ukraine on the European market take the first place, and on the world - the third **[11]**.

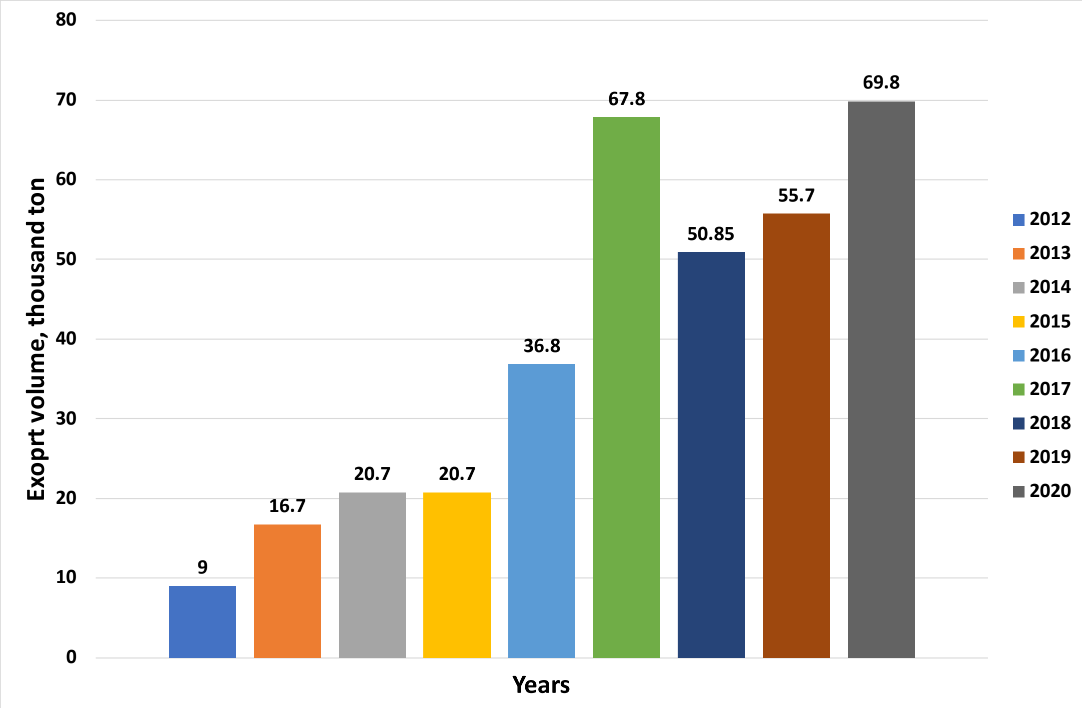


Figure 1 Dynamics of honey exports from Ukraine.

So, both honey and its products are a great way to rejuvenate and revitalize the body. That is why specialists abroad are very interested in honey.

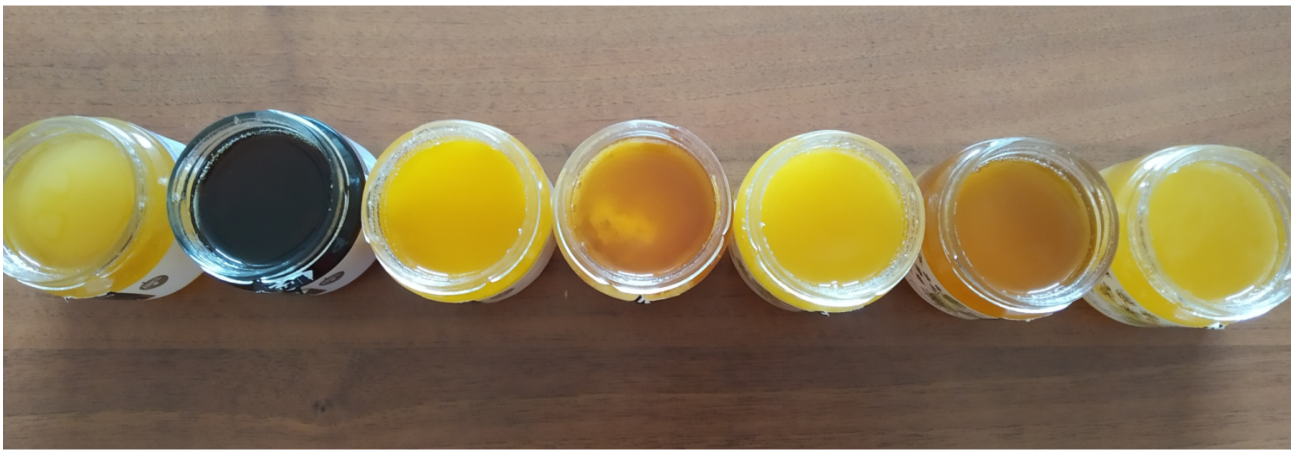
Scientific hypothesis

There is a technology of fermented honey beverages, for which it is necessary to ferment the wort to carry out additional technological operations for its filtration. Our scientific hypothesis is that creating recipes with the addition of different types of honey to honey and preservatives in the form of citric acid and sodium benzoate can reduce the production process and obtain a non-alcoholic product with extended shelf life. The cost of producing such a product will be lower compared to fermented beverages. Therefore, the low cost of the product will allow its use to different segments of the population.

MATERIAL AND METHODOLOGY

Samples

For the study, we used selected samples of honey of seven types and homogenized honey, obtained at the plant engaged in processing and exporting honey to the EU and Canada of the Lviv region.



1 2 3 4 5 6 7

Figure 2 Types of honey for research: 1 – acacia; 2 – buckwheat; 3 – sunflower; 4 – weeds; 5 – goldsmith; 6 – coriander; 7 – linden.

Physico-chemical indicators and indicators of honey safety were determined according to the methods set out in DSTU 4497: 2005 and SOU 01.25-37-373.2005 **[12, 13]**.

Chemicals

Starch (($C_6H_{10}O_5)_n$, (VYMAL), Chernihiv, Ukraine)
Sodium chloride (NaCl, Artemsil State Production Association, Donetsk Region, Ukraine)
Barbituric acid ($C_4H_4N_2O_3 \cdot 2H_2O$, (INTERKHIM), Kharkiv, Ukraine)
Paratuloidin (C_7H_9N, Merck KGaA, Germany)
Isopropanol ($CH_3CH(OH)CH_3$, Ineos Solvents, Germany)
Potassium hexacyanoferrate **(**$K_4[Fe(CN)_6]$, China)
Zinc sulfate ($ZnSO_4 \cdot 7H_2O$, (INFRAKHIM), Yaroslav, Russia)
Acetic glacial acid (CH_3COOH, (LOST LTD), Ivano-Frankivsk, Ukraine)
Sodium hydroxide (NaOH, (Novokhim), Kharkiv, Ukraine)
Hydrochloric acid (HCl, (Novokhim), Kharkiv, Ukraine)
Sulfuric acids (H_2SO_4, (Novokhim), Kharkiv, Ukraine)
Copper sulfate ($CuSO_4 \cdot 5H_2O$, (Novokhim), Kharkiv, Ukraine)
Fermented salt ($KNaC_4H_4O_6 \cdot 4H_2O$, (Novokhim), Kharkiv, Ukraine)
Sodium thiosulfate ($Na_2S_2O_3$, (NOVOSIBKHIMFARM), Novosibirsk, Russia)
Glucose ($C_6H_{12}O_6$, (Novokhim), Kharkiv, Ukraine)
Fructose ($C_6H_{12}O_6$, (Novokhim), Kharkiv, Ukraine)
The citric acid ($C_6H_8O_7$, (Smilyansky sugar factory), Smila, Ukraine)
Sodium benzoate (NaC_6H_5COO, Eastman, Kohtla-Järve, Estonia)

Animals and Biological Material:

For research used: acacia honey; buckwheat honey; sunflower honey; herbal honey; goldenrod honey; coriander honey; linden honey (sold by various manufacturers of Lviv region, Ukraine).

Instruments

Refractometer (IRF-454 B2M, manufacturer, open joint-stock company "KOMZ", Kazan, Russia). To determine the water content in honey.

Laboratory thermometer (TLS-200, manufacturer LLC "Inter-Synthesis", Ukraine).

Photo colourimeter (KFK-3, Altavir Limited Liability Company, Belgorod, Russia).

Flame spectrophotometer (Saturn-4, manufacturer "Inter-Synthesis" Limited Liability Company, Ukraine). The content of toxic elements and radionuclides was determined.

Laboratory Methods

All physicochemical parameters were determined according to DSTU 4497:2005 Natural honey. Specifications.

The mass fraction of reducing sugars was determined using a photocolorimeter using a calibration graph with different concentrations of inverted sugar.

A combined reagent was prepared to determine the diastasis number, which included 0.2 M acetate-buffer solution (pH 5.0), solutions of starch, and sodium chloride. The optical density of the samples was measured on a photocolorimeter at a wavelength of 590 nm against water in a cuvette 10 mm thick.

Solutions of barbituric acid, paratuloidin, and Kerres reagent were prepared to determine hydroxymethylfurfural content. At a wavelength of 550 nm, the optical density of the honey solution was measured relative to the control solution every minute for 6 minutes.

The acidity of honey was determined by titration with sodium hydroxide solution to a pH value of 8.3.

A refractometer was used to determine the water content in the honey.

Toxic elements (lead, cadmium, arsenic) and radionuclides (caesium and strontium) were determined by atomic absorption spectrometry with electrothermal atomization.

Sensory analysis of honey drinks based on a scale was performed.

An analysis of the shelf life of ready-made beverages based on the addition of citric acid and sodium benzoate.

Description of the Experiment

Sample preparation: 7 types of action honey, buckwheat, sunflower, coriander, herbs, linden, and goldenrod were used for the study.

Number of samples analyzed: During the experimental studies, 8 samples were used, 7 of which were described and one sample of homogenized honey, i.e. blended in equal quantities from all species and heated to 35 ºC to determine physicochemical and safety indicators of honey.

Number of repeated analyzes: All measurements were performed 3 times.

Number of experiment replication: The number of replicates of each experiment to determine one value was 5 times.

Design of the experiment: to determine the physicochemical and toxic elements and radionuclides in homogenized honey, equal amounts of honey were mixed and burned to 40 °C, then in chilled honey to 20 °C by known methods determined the indicators presented in Table 1, Table 2 and Table 3. Depending on the amount of added honey, indicators of dry matter content pH values in honey drinks were determined using a refractometer and a pH meter, respectively. 4, 6, 8, and 10 g of honey were added to the samples. During the development of honey drink recipes, the formation of microorganisms in honey samples was visually observed.

Statistical analysis

Mathematical and statistical processing of experimental data was performed to determine the criteria of Cochren, Fisher, and Student. The accuracy of the data was determined using the Cochren test, the adequacy of the mathematical model was checked using the Fisher and Student criteria. Statistical processing was performed in Microsoft Excel 2016. Values were evaluated using mean and standard deviations and subsequently calculated in the statistical program XL Stat. When testing the hypotheses, if the value of p is below a significant level, in the case of XL Stat software from Addinsoft (version 2019.3.2), this value was 0.05, the null hypothesis was rejected, and the alternative hypothesis was confirmed.

RESULTS AND DISCUSSION

Studies of physicochemical and safety indicators of homogenized honey were conducted at the State Research Institute for Laboratory Diagnostics and Veterinary Sanitary Examination, which confirmed compliance with standards and documents presented for honey.

According to the authors **[14]**, the homogenization of natural honey according to the current technological regimes slightly changes its quality indicators and the content of hydroxymethylfurfural. Still, it improves the consumer properties of the product.

As can be seen from Table 1, the content of hydroxymethylfurfural, mass fractions of sucrose, and water in homogenized honey are lower by 22, 23, and 15%, respectively, than the norm.

Table 1 Physico-chemical parameters.

Name of indicator and unit of measurement	Permissible level according to normative documents	Results	Deviation	Conformity mark
The content of hydroxymethylfurfural, $mg.kg^{-1}$	No more than 40.0	29.5	±3.31	Confirmed
Diastasis number, Gotte units	No more than 8.0	29.6	±1.20	Confirmed
Acidity, mEq. NaO $/dm^3$	No more than 50.0	22.8	±1.32	Confirmed
Mass fraction of sucrose (to anhydrous substance),%	No more than 5.0	3.7	±0.41	Confirmed
Mass fraction of reducing sugars (to anhydrous substance),%	No more than 60.0	80.0	±1.59	Confirmed
Mass fraction of water for temperatures of 20 °C,%	No more than 20.0	17.0	±0.22	Confirmed

In scientific works **[15]**, similar studies were conducted to determine the mass fractions of sucrose and water in honey, and the following results were obtained, the figures were lower by 18, 13, and 10%, which in our opinion, may reduce the shelf life of the final product.

In the following manuscripts **[16], [17],** similar studies were conducted to determine the mass fractions of sucrose and water in honey. The following results were obtained, the figures were lower by 20, 10, and 15%.

The diastasis number is 29.6 Gotte units, which indicates the naturalness of honey and its longevity. Some scientific works **[18], [19]** described methods for determining the quality of honey and ways to extend its shelf life and found that at 20 °C, the period of reduced diastase activity is 1480 days, at 25 °C – 540 days, and at 80 °C – only 1.2 hours. However, scientific works **[20], [21]** describe the methods and ways of storing honey and found that the maximum shelf life is only 8 months, after which honey loses its useful properties.

Further research on the development of honey water recipes based on the honey of different types, namely monofloral - acacia, sunflower, buckwheat, coriander, linden, goldenrod, poly flora - weeds, were devoted to finding the optimal amount and different ratio of honey with drinking water. Honey drinks that have shown previous good results. For long-term storage of such beverages, we conduct research with the addition of natural preservatives to prevent the use of substances of chemical origin.

In determining toxic elements and radionuclides in honey, they are either not detected, or the amount is much less than the standard of these substances (Tables 2 and 3).

Table 2 Toxic elements.

Name of indicator and unit of measurement	Permissible level according to normative documents	Results	Deviation	Conformity mark
Mass fraction of lead, mg.kg^{-1}	No more than 0.1	0.046	±0.008	Confirmed
Mass fraction of cadmium, mg.kg^{-1}	No more than 0.03	<0.005	-	Confirmed
Mass of arsenic adol, mg.kg^{-1}	No more than 0.5	<0.01	-	Confirmed

Table 3 Radionuclides.

Name of indicator and unit of measurement	Permissible level according to normative documents	Deviation	Indicator of compliance with radiation safety criteria*	Conformity mark
The content of radionuclides Cs-137, Bk/ kg	No more than 200	<6.34	0.09	Confirmed
The content of radionuclides Sr-90, Bk/kg	No more than 50	<5.84		Confirmed

Note: * – the compliance indicator meets (≤1) radiation safety criteria.

Therefore, from the obtained results, we can say that bee honey homogenized for human consumption on the content of radionuclides meets the State standards "Permissible levels of radionuclides Cs-137 and Sr-90 in food and drinking water" approved by the Ministry of Health of Ukraine, order from 03.05 .2006, No 256 with changes; in terms of physicochemical parameters complies with the Directive of the Council of the European Union 2001/110/EC, 396/23/EC of December 20, 2001; complies with the content of toxic elements following "Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and products of animal origin"; DSTU 4497:2005.

Many scientific manuscripts **[22], [23]** have conducted studies on the comparative analysis of honey samples, but the authors only determine organoleptic indicators. In our opinion, this method can not be effective, and the next step should be to create an ISO standard for honey from Europe or USA.

A series of similar experiments are described in the following works **[24], [25]**. Still, the authors of the above scientific works conduct a comparative analysis of honey samples according to GOST 19792-2017 **[26]** and TU 01.49.21-077-37676459-2017 **[27]**. Still, in our opinion, these methods can not be used for honey from Europe, Asia or Africa.

To develop recipes for honey water, we used various blends of honey to obtain a pleasant drink. In figure 3, you can see the change in dry matter content in honey water depending on the type of honey that was added to a drink and the amount of honey in 100 g of water.

From the diagram, you can see the increase in dry matter content in samples of beverages with a higher content of honey but different amounts of dry matter content within the same amount of honey. Based on physicians' recommendations on the use of the daily norm of honey, we selected samples corresponding to 6 g of honey in 100 ml of water. For this variant, the highest honey content corresponds to a mixture of honey from sunflower and coriander, which indicates a smaller amount of water in them compared to the samples of acacia + buckwheat and herbs + goldenrod + linden.

Similar scientific researches which are connected with the preparation of honey drinks with various combinations of samples of honey are described in the following scientific works **[28]**, **[29]**. Some combinations of samples can be incompatible, and their combination will be of no use.

Studies related to the preparation of honey drinks with different samples of honey-based on fruit juices are described in the following scientific papers **[30]**, **[31]**. Still, in our opinion, some combinations of samples may be incompatible, and their combination may lead to the formation of harmful microflora in the finished product.

Drinking water without additional treatments was used to prepare drinks. The pH of this water was 7.20 and distilled – 7.55. Similar studies have been conducted in scientific works **[32]**, **[33]**, but various fruit juices, milk, dairy products, and pre-purified water were used to prepare beverages.

A similar series of experiments were conducted by the authors of the following manuscripts **[34]**, **[35]**. Still, for the preparation of honey drinks used a variety of dairy products, these are quite interesting experiments. They have no recommendations for shelf life and no organoleptic evaluation.

Since honey has an acidic environment accordingly, the pH value of beverage samples decreased due to the addition of honey to water. Figure 4 shows that the minimum pH value, regardless of the amount of honey added to the water, corresponds to honey samples from sunflower and coriander. The maximum pH value is obtained by adding a mixture of goldenrod herbs and linden.

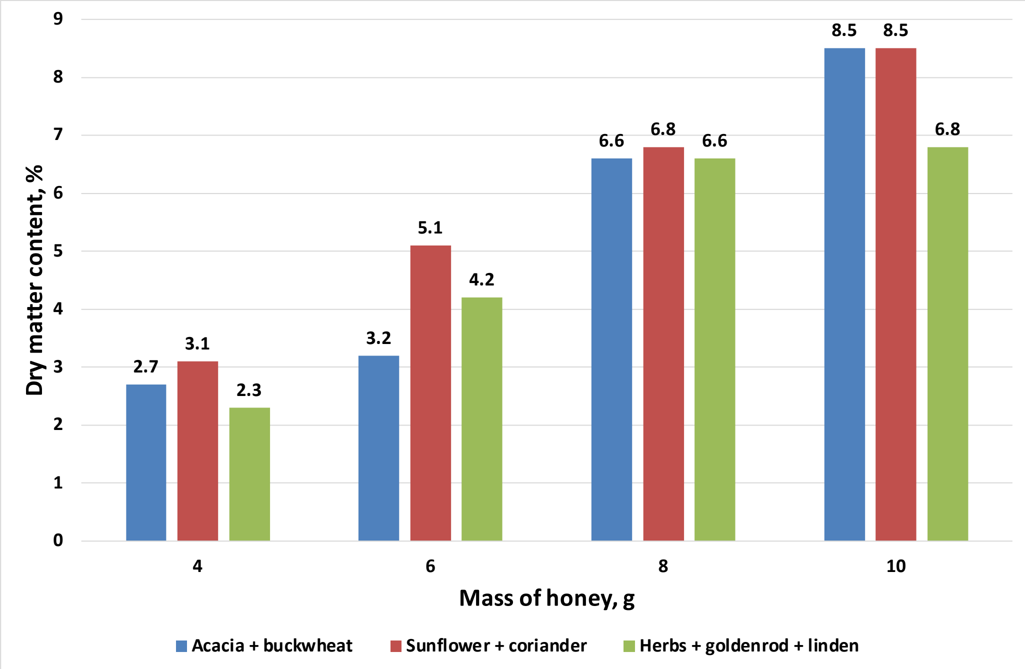


Figure 3 Change in dry matter content in different samples of honey water.

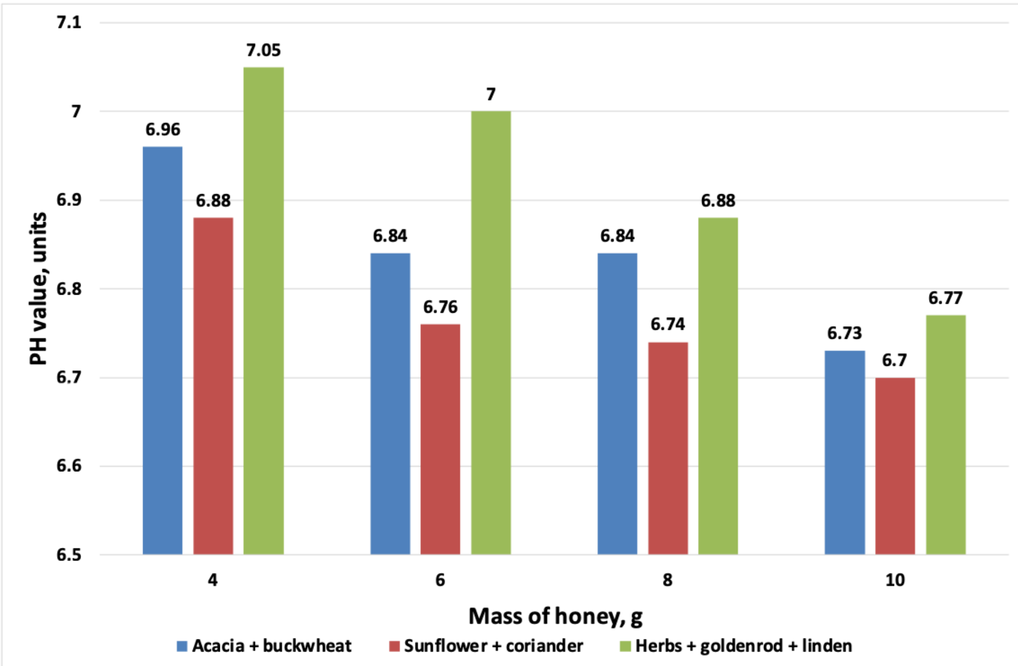


Figure 4 The effect of pH on the amount and types of honey.

The smaller the pH value, the better the honey water will be stored because the acidic environment harms the development of microorganisms. Therefore, our further research was experimenting to find the shelf life of such honey water without additional components at room temperature and with the addition of citric acid.

Black mould appeared after the storage of honey water samples at a temperature of 18 ºC on the seventh day (Figure 5). However, as can be seen from the photo, when adding 6 g of honey in 100 mL of water, this mould is absent, and for other samples of honey, visible colonies of microorganisms.

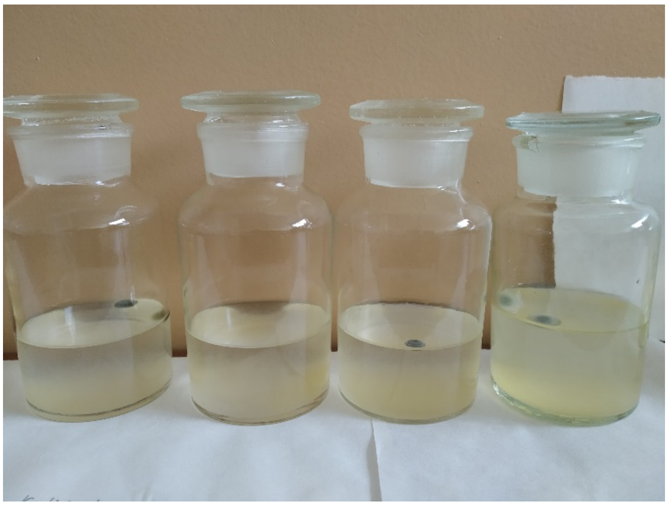


Figure 5 Presence of black mould in honey water samples.

When added to the samples of honey water, which contained 6 g of honey, 0.5 and 1.0 g of citric acid (Figure 6) compared with the control test on the tenth day, yeast appeared (Figure 7), which indicates the elongation shelf life of honey drink for three days longer than without the use of citric acid. Moreover, more yeast can be seen visually with less added citric acid.

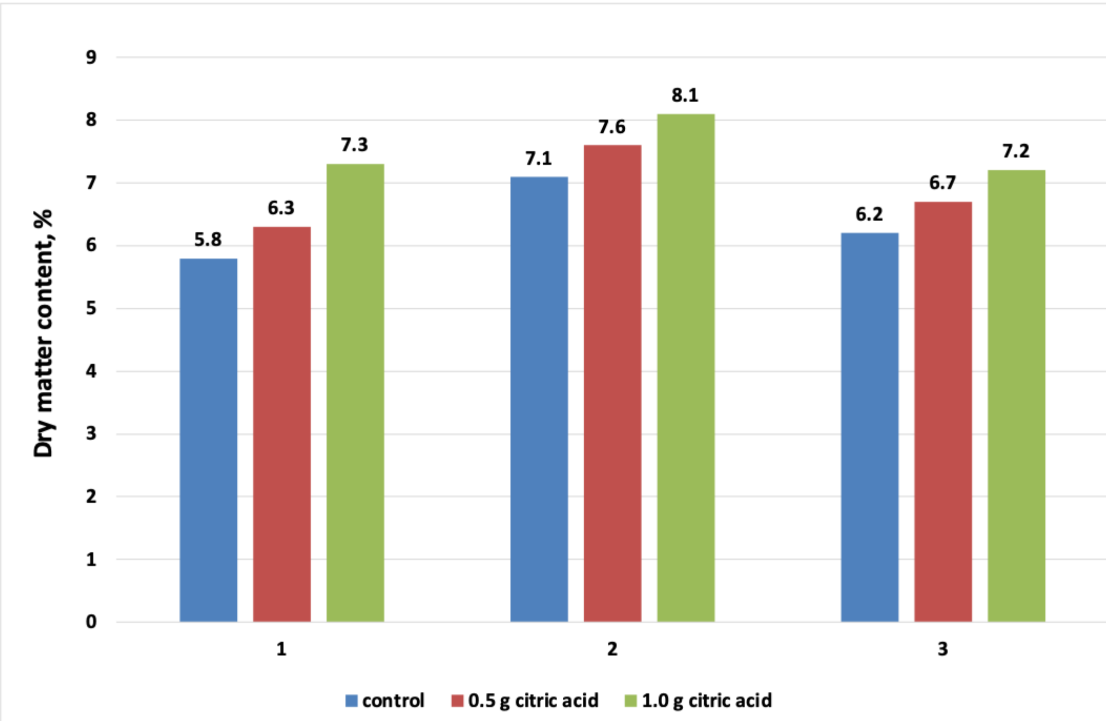


Figure 6 Change in dry matter content with the addition of citric acid: 1 – acacia and buckwheat; 2 – sunflower and coriander; 3 – weeds, goldenrod, and linden.

The citric acid in the drink acts as a regulator of acidity and, therefore, extends its shelf life. But this is not enough to increase the shelf life of the drink. Consequently, it is impossible to produce such honey water without using a preservative because, in addition to the formation of microorganisms, there is also a fermentation process due to free glucose in honey.

The authors of the following scientific works **[36], [37]** conducted many similar studies, which developed recommendations for extending the shelf life of honey and products with its components through the use of various storage methods, which we believe should be better studied.

Scientific works **[38], [39]** describe a series of experiments that investigated various technologies and equipment to extend the shelf life of honey and products with its components using a variety of preservatives that can inhibit the beneficial properties of the final product.

Figure 7 Formation of yeast during storage of honey water.

Formulations of honey water based on water, honey, and preservative - sodium benzoate in the amount of 0.1% by weight of the drink to extend the shelf life was developed.

Figure 8, Figure 9 and Figure 10 show 4 samples of honey drinks with the same amount of honey from 4 to 10 g (4, 6, 8, and 10 g) per 100 g of water (from left to right). Thus, in Figure 8 you can show samples of honey drinks using acacia and buckwheat, in Figure 9 – sunflower and coriander, and in Figure 10 – a mixture of honey from herbs, goldenrod, and linden. As can be seen from Figure 8, Figure 9 and Figure 10, the colour of honey drinks is different because the colour itself is different for different types of honey. The most intense colour corresponds to the honey drink "Fantasy" because buckwheat honey has a dark brown hue. Linden and goldenrod honey are the lightest, so the drink "Honey Mixture" is also not rich in colour.

Figure 8 Development of the recipe for the drink "Honey Fantasy" based on acacia and buckwheat honey.

Figure 9 Development of the recipe for the drink "Honey Delight" based on sunflower and coriander honey.

Figure 10 Development of the recipe for the drink "Honey Mix" with the addition of honey from herbs, goldenrod, and linden.

Organoleptic evaluation of the created recipes of honey drinks is carried out. According to the results, the obtained drinking honey fully meets the standard, is characterized by transparency, developed light, characteristic of this type of honey, smell and taste, pleasant aftertaste, the bouquet is tender and developed (Table 3).

Table 3 Tasting evaluation of honey water.

Indicators	Characteristic	Rating (points)
Transparency	Crystal clear	0.5
Colour	Full compliance with the default	0.5
Bouquet	Thin and developed	4
Taste	Very thin and developed	4
Default	Full compliance with the default	1
Overall rating	High-quality drink	10

According to research, it is established that for 1000 kg of finished honey drink, you need 993 kg of water, 6 honey, and 1 kg of sodium benzoate.

The drink can be made on an industrial scale and stored in a closed container for one year when using such a formulation.

This research does not end this work because there are still some questions about the use of water for drinking - what should be this water, whether it is possible to use spring water, what other ingredients it is desirable to add to the drink to use a useful product and so on.

CONCLUSION

Studies have been conducted to determine the physicochemical, toxic, and radionuclides in homogenized honey, a mixture of acacia, buckwheat, coriander, sunflower, weeds, goldenrod, and linden. These studies have confirmed that honey meets the state standard requirements for natural honey. Based on the types mentioned above of honey, studies were conducted to determine the dry matter content and pH value when adding different amounts of honey to create recipes for honey drinks. Studies with the addition of citric acid in amounts of 0.5 and 1.0% by weight of the drink did not give positive results in extending their shelf life. It is recommended to add the preservative sodium benzoate in 0.1% by weight to the beverage to extend the shelf life.

Based on seven types of honey, recipes for honey drinks have been developed. The production of which on an industrial scale will expand the range of non-carbonated products and reduce the duration of the technological process and the cost of finished drinks.

It is recommended to add the preservative sodium benzoate in 0.1% by weight to the beverage to extend the shelf life.

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