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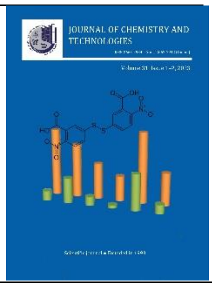

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INNOVATIVE WATER PREPARATION TECHNOLOGY FOR PRODUCTION OF KOMBUCHA FERMENTED BEVERAGE

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Abstract

The article presents the results of theoretical and experimental research on improvement of water preparation technology for non-alcoholic production of kombucha fermented drink. Complex action of the studied materials with adsorptioning, ion exchanging and redoxing properties provides the water conditioning, which allows improving the organoleptic indicators of the final product and increases its biological value. The expediency of using in the technology of preparation water for making kombucha drink has been established for clinoptilolite, rock crystal and activated carbon. While treating water from various sources with the proposed materials it's overall softness and the total iron content were decreased, the content of biologically active substances in the final drink increased and organoleptic characteristics improved. The content of polyphenols substances in the final drink increased on the average 1.6 times compared to the untreated artesian water and the water from centralized water supply network. It was found that the high water hardness had a negative effect on the fermentation caused by the *Medusomyces gisevii* yeast culture and it made the organoleptic qualities of the final drink worse. The correlation between water indicators, wort fermentation dynamics and organoleptic evaluation of final drinks had been defined.

The highest organoleptic values had been got by the samples of the drink prepared using water treated with clinoptilolite, activated carbon and rock crystal.

Keywords: water; non-alcoholic fermented drinks; kombucha; water treatment; clinoptilolite; rock crystal; activated carbon.

ІННОВАЦІЙНА ТЕХНОЛОГІЯ ПІДГОТУВАННЯ ВОДИ ДЛЯ ВИРОБНИЦТВА ФЕРМЕНТОВАНОГО НАПОЮ КОМБУЧА

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Анотація

У статті наведено результати теоретичних та експериментальних досліджень з удосконалення технології підготовки води для виробництва безалкогольного ферментованого напою комбуча. Комплексна дія досліджених матеріалів із адсорбційними, іонообмінними та окисно-відновними властивостями забезпечує кондиціювання води, що дозволяє покращити органолептичні показники готового продукту та підвищує його біологічну цінність. Встановлено доцільність використання клиноптилоліту, гірського кришталю та активованого вугілля у технології підготовки води для приготування комбучі. При обробці води з різних джерел запропонованими матеріалами зменшувалась її загальна жорсткість, вміст загального заліза, збільшувався вміст біологічно активних речовин в готовому напої, покращувались органолептичні характеристики. В готовому напої збільшувався вміст поліфенольних речовин в середньому у 1.6 рази у порівнянні з використанням необробленої води з артезіанської свердловини та централізованої мережі водопостачання. Встановлено, що висока жорсткість води негативно впливала на зброджування сусла культурою *Medusomyces gisevii* та погіршувала органолептичні якості готового напою. Визначено кореляцію між показниками води, динамікою бродіння сусла та органолептичною оцінкою готових напоїв. Найвищу органолептичну оцінку отримали зразки напою, що приготовлені з використанням води, обробленої послідовно клиноптилолітом, активованим вугіллям та гірським кришталем.

Ключові слова: вода; безалкогольні ферментовані напої; комбуча; водопідготовка; клиноптилоліт; гірський кришталь; активоване вугілля.

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Introduction

The functional drinks based on the natural raw materials take an important place in the health diet. These drinks are intended for consumption by the children, athletes, elderly people, etc. Investigations were aimed to deter-mine the human organism needs of different cate-gories and their production optimization [1–3].

The main trends that had a great impact on the development of the functional drinks segment are convenience, enjoyment, ethnic integration, traditions and health benefits. Over the past 10 years the consumption of the functional beverages by the population of Europe had increased by 55 % and it reaches almost 130 dm 3 per year [1–3]. According to Zenith International agency research, the market of functional drinks in the Western Europe by 70 % consists of sports, energy, nutraceutical and the tea raw materials drinks. At the same time, the largest growth rates are demonstrated by the market of cold teas and the cold tea based drinks, including kombucha [4].

According to Zion Market Research, the global kombucha market in 2016 was valued at approximately 1.062 billion US dollars and it is expected to reach approximately 2.5 billion in 2022 with an annual increase of about 25.0% [5].

This drink is relatively new for the countries of Europe and North America. However, in China the history of its use goes back more than two thousand years. In Ukraine this drink is at the household level, it is known as "tea mushroom". Kombucha is recognized around the world for its detoxifying, energetic and general health-giving properties. It is also used for medicinal purposes [6; 7].

Externally, the culture of Medusomyces gisevii has the appearance of a thick multi-layered film of yellowish-brown color. The young culture grows from above with forming the layering. Fermented wort based on sugar and tea infusion is an aromatic, refreshing drink with a low alcohol content, lightly carbonated, pleasantly acidic-sweet taste, which depends on the duration of fermentation and the composition of tea raw materials [7; 8].

It is believed that kombucha has the healing and preventive properties: it improves the activity of the gastrointestinal tract, inhibits putrefactive microflora, strengthens intestinal peristalsis and also normalizes stomach acidity.

Kombucha can be useful for elderly people suffering atherosclerosis; its regular using reduces the level of cholesterol in the blood, headaches of a neurological nature and pain in heart, it also significantly helps with stone-kidney disease [9–11].

The chemical composition of the drink is the result of wort fermentation by symbiotic cultures of microorganisms. Medusomyces gisevii contains the yeast Saccharomyces ludwigii, Saccharomyces cerevisiae, Brettanomyces bruxellensis, Candida stellata, Schizosaccharomyces pombe, Torulaspora elbrueckii, Zygosaccharomyces fermentati, Zygosaccharomyces bailii, etc., bacteria of the genus Acetobacter, etc. [12; 13].

The basis for the production of kombucha is sugar, tea and water. Its indicators affect on the quality of the drink and the course of the technological process. The extractive substances of tea raw materials are the active chemical compounds that interact with the components of the wort.

The water supplied to food productions contains as a rule elevated amount of iron, calcium and magnesium salts, it has an increased color. Food productions make the strict requirements to water. That's why they include water treatment lines ensuring the necessary technological quality of water [13].

Enterprises producing soft drinks use water from centralized water supply networks or artesian wells. In the first case, the water is already brought to potable condition, and in the second one, that occurs more often, it may not meet such requirements. Besides, there are also additional requirements for processing water in non-alcoholic production as to hardness and total iron content indicators [14]. However, no such requirements have been developed for the production of non-alcoholic fermented beverages, in particular kombucha.

In many cases, as well as for blending soft drinks, water softened on sodium-cationic filters is used, which does not fully meet the requirements of biotechnological processes. Therefore, it is urgent to improve the technology of water preparation with various types of natural materials that are effective and convenient for practical use. Modern water purification technologies involve the use of natural and artificial minerals to solve various problems. Sorption processes are implemented by using activated carbon and its analogues. For the same purpose zeolites are used, as well as a filter material [15].

There are various methods of preparing water, including using mechanical filters with backfills of natural minerals (quartz sand, gravel) [14]. At conditioning water for the production of fermented beverages using such filtering is mandatory. As a result of such treatment, water is freed from mechanical impurities, colloidal suspension and sediment. However, such processing does not completely ensure the quality of prepared water according to organoleptic, physicochemical and toxicological indicators. Therefore, it is relevant to improve the method of water treatment using new effective natural materials, in particular minerals. Thanks to strictly defined sizes of pores and internal cavities, they are effective sorbents of organic and inorganic substances. Such materials have the ability to improve the organoleptic and physicochemical indicators of water, to ensure its structuring and enhance the health-improving effect of the final drink [15]. Modern water purification technologies involve the use of natural and artificial minerals to solve various problems. Sorption processes are implemented by use of activated carbon and its analogues. For the same purposes, as well as filter material, zeolites are used [15]. Zeolites had no practical application for a long time. Later it was established that they can be used in many industries due to their unique properties. Today more than forty structural types of zeolites are known; the most widespread of them are clinoptilolite, heylandite, phillipsite, lomonite, mordenite, erionite, shabasite, analcime [16].

One of the world's largest deposits of zeolites is located in Ukraine, in the village of Sokyrnytsia, Khust district, Transcarpathian region. Clinoptilolite from this deposit contains 85...90% of the main component. Transcarpathian clinoptilolite in the oxide version has the following composition, %: SiO_2 – 67.29; TiO_2 – 0.26; Al_2O_3 – 12.32; Fe_2O_3–1.6; FeO – 0.25; MgO – 0.99; CaO – 3.01; Na_2O – 0.66; K_2O – 2.76; H_2O – 10.90 [15]. They have low cost, unique and useful technological properties: selective (cation exchange, molecular sieve), sorption (primarily adsorption) and catalytic, caused by the features of their crystal lattice and chemical composition. These are water-aluminosilicate minerals of a framework structure, which makes possible using them as effective filter material for water purification [17; 18].

Activated carbon is a highly porous carbon sorption material and a porous adsorbent made of organic materials containing carbon [19].

Activated carbon is produced of wood (birch, oak, beech, hornbeam), coconut shell, peat (peat coke), coal coke, less often of walnut shell, olive pits, apricot pits and others. It is used in water treatment: powdered, granular (shredded or granulated), as well as fibrous [19; 20]. Activated carbon is the most commonly used adsorbent for industrial water treatment due to the presence of a large number of micropores (less than 2 nm) and mesopores (2...50 nm), a significant adsorption surface area (500...1500 m^2/g) and surface reactivity [21]. In modern water purification systems the use of Silcarbon K835 activated carbon is quite widespread. It is obtained from coconut shell by pyrolysis. It has a high density and mechanical strength, which makes possible to carry out multiple regeneration in carbon filters with the provision of high organoleptic indicators of water, in particular transparency, color, smell, taste and aftertaste [22–24].

Rock crystal, as well as rauchtopaz, morion, smoky diamond, smoky crystal, cairn gorm, Scotch stone, is a variety of the natural mineral quartz, belonging to the class of oxides and hydroxides. Rock crystal is used in liquor-vodka production for the final processing of the water-alcohol mixture and coloring of its tasting evaluation. It is not used in water treatment schemes, in particular for the production of fermented beverages [25].

Therefore, the use of natural minerals, in particular zeolites and rock crystal, in the purification of natural waters will allow not only removing unwanted substances, but also ensuring the necessary salt composition of water without the use of chemical reagents. This is the prerequisite for their use in water treatment in fermented beverage technologies. The additional use of activated carbon and rock crystal is a prerequisite for organoleptic stabilization and improvement of microbiological and physicochemical indicators.

Materials and methods

The researches used: Medusomyces gisevii consortium according to passport data, drinking water from the centralized water supply of Kyiv city and the artesian wells according to DSanPiN 2.2.4-171-10 [26], white sugar according to DSTU 4623-2006 [27], clinoptilolite from the Sokyrnytskyi deposit, activated carbon Silcarbon K835, rock crystal according to the current regulatory documentation.

The research methods generally accepted in the beer-non-alcoholic industry of the food industry were used in the present work. Water treatment was carried out on a laboratory installation, which included a pressure collector with a capacity of 10 dm^3, a filter with a capacity of 1.0 dm^3 with activated carbon and filter materials, a receiving collector with a capacity of 10 dm^3. Demineralized water was obtained by passing water through a distiller.

To prepare wort, raw and treated drinking water was used in the sequence of clinoptilolite, activated carbon and rock crystal at a speed of 8...15 m/h.

Samples of water for making wort are:

1 – water from an artesian well;

2 – water from an artesian well after boiling for 30 minutes;

3 – water from an artesian well after successive processing through mechanical filters with clinoptilolite, activated carbon and rock crystal;

4 – tap water from the centralized water supply network (Kyiv);

5 – water from the centralized water supply network (Kyiv) after boiling for 30 minutes;

6 – water from the centralized water supply network (Kyiv) after successive processing through mechanical filters with clinoptilolite, activated carbon and rock crystal;

7 – demineralized water.

Methods of determining water parameters are presented in the Table. 1.

Table 1

Methods of determining water indicators

Indicator	Research method	The essence of the method	Literary source
pH, units pH	Potentiometric	Determination of the hydrogen ions' activity by an ion-selective electrode	DSTU 4077-2001
Total hardness, mmol/dm^3	Titrimetric	The formation of a complex compound of trilon B with calcium and magnesium ions during titration	DSTU ISO 6059-2003
Alkalinity, mmol/dm^3	Titrimetric	Determined by the method of neutralization using color indicators	DSTU ISO 9963-1:2007
Chlorides, mg/dm^3	Titrimetric	Titration with silver nitrate using chromate as an indicator (Mohr's method)	DSTU ISO 9297:2007
Residual free chlorine, mg/dm^3	Titrimetric	Oxidation of iodide with residual chlorine to iodine with sodium thiosulfate titration	DSTU ISO 7393-3
Total iron, mg/dm^3	Photometric	Interaction of ferrous iron with 2,2-bipyridyl to form a complex compound	DSTU ISO 6332-2003

The wort was prepared by adding sugar syrup and tea infusion to water to a dry matter concentration of 7.0...7.2 %. The culture of *Medusomyces gisevii* in the amount of 3 % was added to the finished wort. The duration of fermentation was 14 days. After fermentation, the fermented wort was cooled, and the culture of microorganisms was removed.

Physicochemical indicators of wort and kombucha were determined by physicochemical and organoleptic methods. Mass fraction of dry substances according to DSTU4855:2007 and total acidity were determined by titration with alkali in the presence of phenolphthalein according to DSTU 7102:2009. The content of caffeine in wort was determined by the photometric method based on the hydrolytic oxidation of caffeine into tetramethylpurpuric acid and the following photometric measurement of its solution color intensity at the wavelength 540 nm according to DSTU4102-2002. An expert group consisting of 5 people was interviewed for the assessment of organoleptic indicators. The tasters were in the same conditions in a room with an air temperature of 20±2 °C. The main descriptors for evaluating the quality of drink samples were: intensity and harmony of aroma, sweetness, acidity, fullness of taste, intensity of saturation with carbon dioxide. The research was carried out according to DSTU7099:2009.

Results and discussion

During the production of kombucha it is necessary to take into account the physical and chemical parameters of the water, in particular, the hardness, the content of iron and chlorides, which affect the course of the fermentation process of the wort, the quality indicators and the usefulness of the final product.

On the basis of research on the effect of the prepared water indicators on the quality of bread kvass [28, 29], a study was carried out as to possibility of using the prepared water in the proposed method in the technology of fermented drink kombucha using the *Medusomyces gisevii culture*.

Physico-mechanical properties of clinoptilolite, rock crystal and activated carbon are presented in the Table. 2.

Table 2

Physico-mechanical characteristics of sorption and filter materials

Indicator	Name of the material		
	Rock crystal	Clinoptilolite	Activated charcoal
Bulk density, g/dm^3	1300± 64.8	246± 11.8	450± 21.6
Humidity, %	4±0.2	4±0.2	5±0.2
Mechanical strength, %	98.5±4.7	95±4.5	96±4.6
Granulometric composition, mm	1…2.5	3…7	1…2.5

The mechanical strength of the studied material samples was more than 95%, which helps to increase their operating term and the number of regenerations, to shorten the start-up period, and to reduce the consumption of water and reagents for washing [28]. The average particle size of clinoptilolite is 2.9 times bigger than that of rock crystal and activated carbon, which determines its use at the first stage of water treatment.

The technology of fermented drinks production, as a rule, does not involve additional water treatment. However, the chemical composition of the source water has a significant impact on the technological process and indicators of the final product. Therefore, compliance of water only with drinking requirements [26] is insufficient.

The *Medusomyces gisevii* consortium is an unconventional culture for fermented beverages both in terms of the species composition of microorganisms and the nature of cultivation. So, it was appropriate to carry out research on the influence of the chemical composition of water on the indicators of wort fermentation by this culture. To reduce water hardness, clinoptilolite was used, which allows not only adjusting the calcium and magnesium content, but also reducing significantly the ammonium content and alkalinity. This is explained by the peculiarities of its framework structure, which is a tetrahedron, the vertices of which form eight-membered rings, creating channels in the structure of the mineral. Molecules of "zeolite water" and cations of alkaline (Na^+, K^+), and alkaline earth (Ca^{2+}, Mg^{2+}) metals are placed inside the channels. Having a big number of entrance windows on the surface, a structure permeated by channels, a complex of cations inside, clinoptilolite can be used as a molecular sieve with the substitution of cations capable of entering through molecular windows with an entrance size of 3.5...4.8 A° [17, 28].

To reduce the content of organic substances and improve organoleptic indicators, sorption water purification with microporous activated carbon was used.

For the purpose of structuring water, adjusting the redox potential and disinfection, rock crystal was used.

The researchers used water from different sources and treatment methods. The chemical composition of the studied water samples are presented in the Table 3.

Table 3

Chemical composition of the studied water samples

Water indicator	Sample №						
	1	2	3	4	5	6	7
pH	7.27	7.7	7.75	6.85	7.0	7.4	6.5
General stiffness, mmol/dm³	11.8 ±0.53	10.2 ±0.5	6.6 ±0.3	4.1±0.18	2.2 ±0.08	1.0 ±0.05	0±0.01
Total alkalinity, mmol/dm³	0.71±0.02	0.35±0.01	0.61±0.02	0.38±0.01	0.36±0.01	0.34±0.01	0±0.01
Chlorides, mg/dm³	92.1±4.6	46.7±1.9	3.1±0.14	41.2±1.86	23.1±0.94	2.1±0.1	0.02±0.01
Residual free chlorine, mg/dm³	0±0.01	0±0.01	0±0.01	0.4±0.01	0±0.01	0.05±0.01	0±0.01
Total iron content, mg/dm³	0.17 ±0.01	0.17±0.01	0.04±0.01	0.1±0.01	0.1±0.01	0.03±0.01	0.01±0.01

It was established that in the process of preparing water from various sources, the proposed materials reduce the overall hardness of water. It was established that water treated in the sequence of clinoptilolite, activated carbon and rock crystal had the most acceptable physicochemical parameters. The water acquired a pleasant taste without unpleasant odors. The total hardness of water from an artesian well decreased by an average of 1.8 times, from a centralized water supply network by 2 times, the content of total iron by 23.5 % and 30 %, respectively.

Active acidity is one of the most important indicators of water quality, which significantly determines the nature and speed of chemical and biological processes, it also determines the acidity or alkalinity of water.

Calcium and magnesium salts, characterized by water hardness, interact with organic acids, reduce their content in the final drink and form complex compounds that cause turbidity and reduce the acidity of the drink. In addition, they directly affect the content of biologically active substances in the drink.

The influence of the studied water samples on the content of polyphenolic substances in the wort was studied (Fig. 1).

It was established that the physicochemical parameters of water influenced significantly the content of polyphenolic substances in the wort. It was established that the content of polyphenolic substances in the wort increased with the decrease in water hardness.

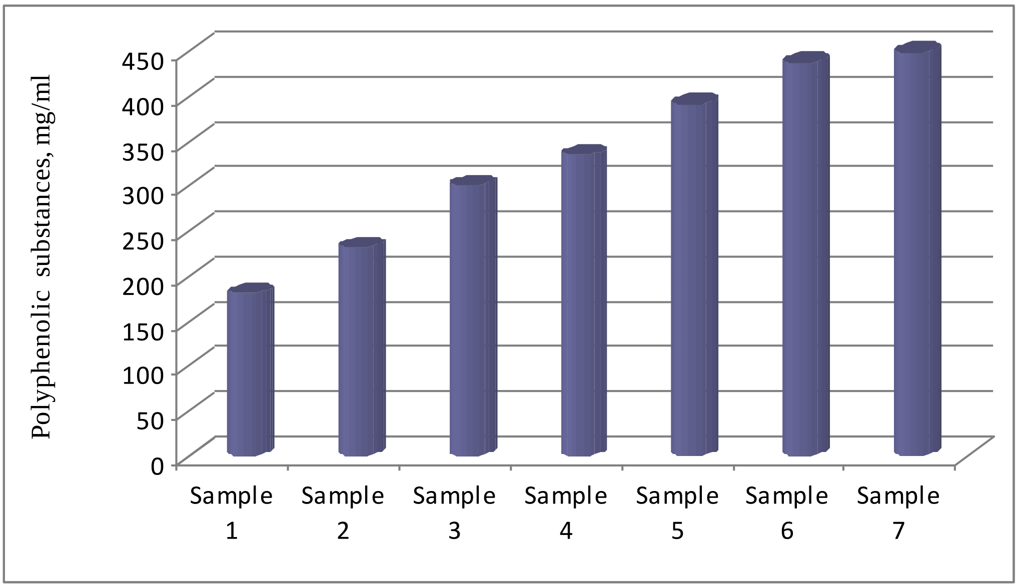
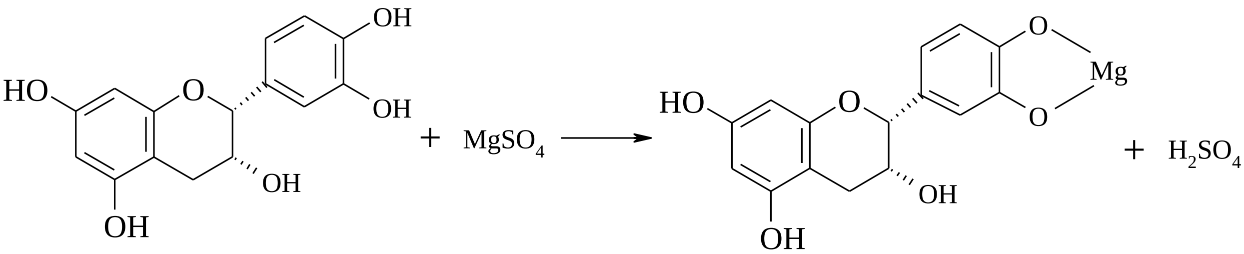


Fig. 1. Content of polyphenolic substances in wort

Thus, while preparing water by the proposed method in samples 1 and 4, compared to samples 3 and 6, the content of polyphenolic substances was 1.8 and 1.4 times higher, respectively, and while comparing the first and seventh samples, their content in the wort was 2.8 times higher.

The effect of general water hardness on the content of polyphenolic substances in the tea solution is due to the formation of coordination bonds with calcium and magnesium salts. For magnesium sulfate the reaction occurs as follow:



To preserve the maximum amount of polyphenolic substances in the wort, and, accordingly, to increase the biological value of the fermented drink, the total hardness of the water for the production of kombucha should not exceed 2.0 mmol/dm^3.

The maximum allowable concentration of total iron content in drinking water according to existing requirements is 0.2 mg/dm^3 [26]. The interaction of phenolic substances with iron occurs similarly to the formula given above. Iron, like hardness salts, affects significantly the biological value of kombucha and its organoleptic indicators. These metals are able to bind molecules of polyphenolic substances in tea, which leads to the formation of complexes causing clouding and precipitation of substances, and, accordingly, deterioration of the organoleptic evaluation.

From the point of view of the functional impact of kombucha on the human body, in addition to polyphenolic substances, vitamin C and caffeine are of great importance. They have antioxidant properties, low redox potential, and therefore are easily oxidized.

Fig. 2 shows the content of caffeine in wort while using the studied water samples.

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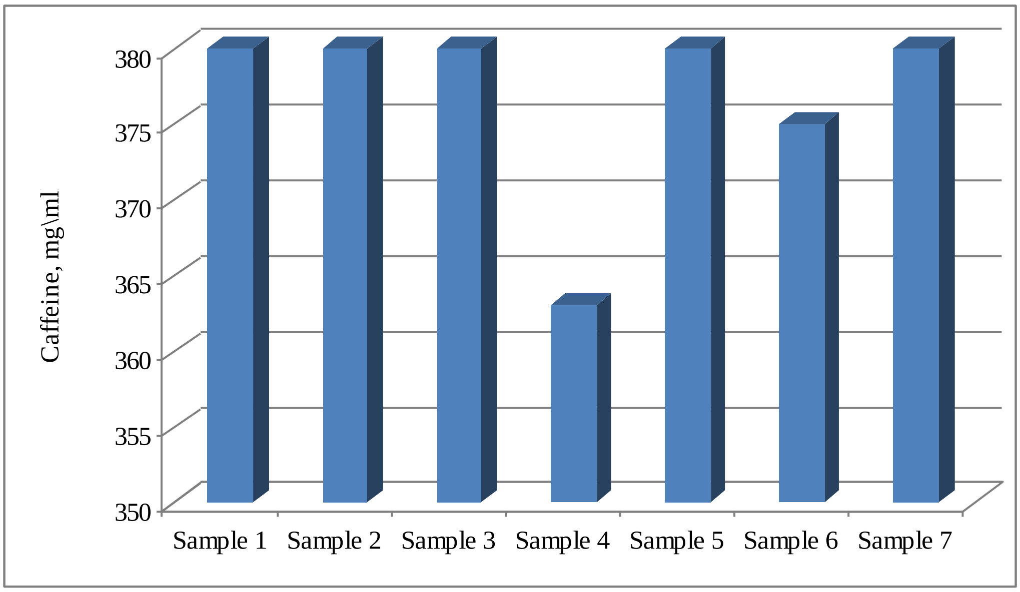
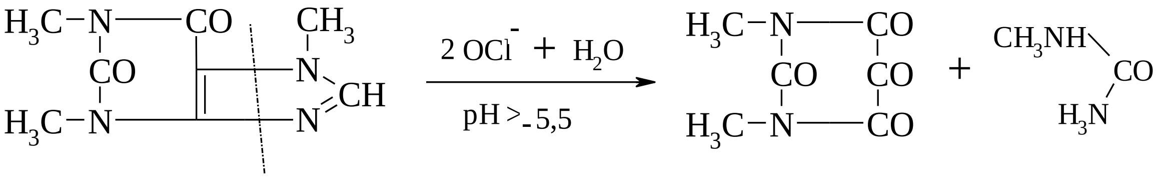


Fig. 2. Caffeine content in wort

It was found that compared to other samples, the caffeine content was significantly different in the sample 4, which can be explained by the presence of residual free chlorine in tap water.

In the process of oxidation caffeine loses its biological activity and decomposes into dimethylsiloxane and methylurea according to the next formula



Caffeine and related xanthines in the tea solution have the ability to stimulate the synthesis of a cellulose film by Acetobacter bacteria, which strengthens the connection between bacteria and yeast and has a direct effect on the fermentation process. Fig. 3 and 4 show the dynamics of wort fermentation while using the investigated water samples.

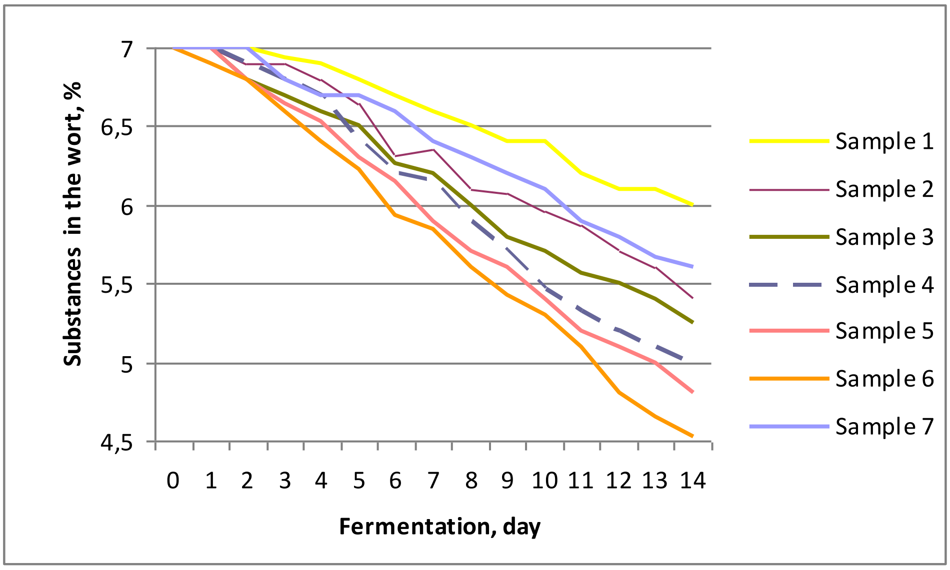


Fig. 3. Dynamics of changes in the content of dry substances in the wort during the fermentation process

It was established that the most intensive fermentation process occurred from the fourth to the eighth day, which can be explained by the logarithmic phase of growth of yeast cells as a component of the Medusomyces gisevii consortium. The wort of samples 5 and 6 fermented the most, which is at an average 16.5% more compared to other samples.

The slowest wort fermentation was observed in the first sample and on the 14th day, the decrease in dry matter content was at an average 23.3 % less compared to other samples.

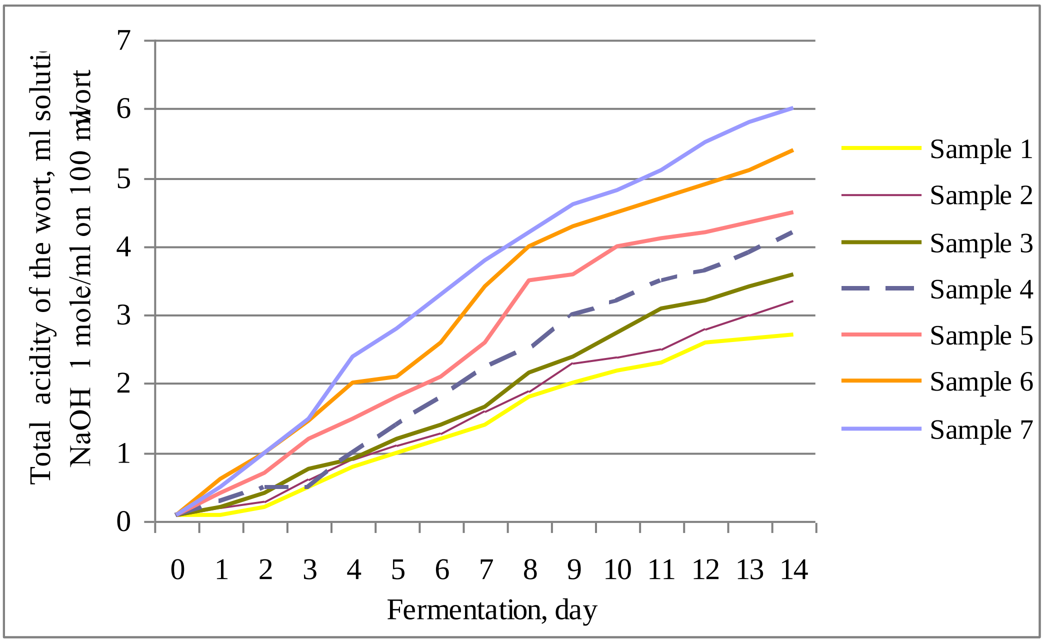


Fig. 4. Dynamics of changes in the total acidity of the wort during the fermentation process

The total acidity of the wort of all samples increased during fermentation, which is explained by the accumulation of mainly acetic and gluconic acids formed by the oxidation of ethanol and the aldehyde group of glucose, respectively. The acidity increased most intensively for the seventh sample, which can be explained by the absence of hardness salts in the source water.

The general trend of the negative effect of hardness salts on fermentation dynamics was observed for other samples as well. The fermentation process was negatively affected by the increased content of residual free chlorine (sample 4), especially in the first days of fermentation. This can be explained by the content of residual chlorine in the water of the centralized city network.

Given that one of the main criteria of the soft drinks quality determining their consumer properties are their organoleptic indicators.

The Table 4 shows the organoleptic evaluation of final drinks obtained using the studied water samples.

Table 4

Organoleptic indicators of the final drink

№ sample	Organoleptic assessment, points
1	8.0 (unsatisfactorily)
2	8.8 (unsatisfactorily)
3	12.8 (satisfactorily)
4	16.8 (good)
5	18.0 (perfectly)
6	18.5 (perfectly)
7	12.5(satisfactorily)

A correlation between water parameters, dynamics of wort fermentation and organoleptic evaluation of final drinks was established. Thus, the lowest score was given to drinks prepared on water of samples 1 and 2. The intermediate position was taken by samples 3, 7, 4. Samples 5 and 6 were recognized as the best. The highest score was given to the drink prepared on water treated in the sequence clinoptilolite, activated carbon and rock crystal. The exceptional position of sample 7 (low organoleptic evaluation at high fermentation rates) is explained by the use of demineralized water.

Conclusions

1. The expediency of using clinoptilolite, rock crystal and activated carbon in water treatment technology for the production of kombucha has been established.

2. While treating water from various sources with the proposed materials, its overall hardness, total iron content, increase the content of biologically active substances in the wort, improve

the organoleptic indicators of the final drink, increase the content of polyphenolic substances by an average of 1.6 times compared to the use of untreated water.

3. Based on the results of studies of the wort fermentation dynamics, it was established that high water hardness negatively affects the fermentation process by Medusomyces gisevii culture and worsens the organoleptic qualities of the final drink. The wort was fermented most completely using water from a centralized city network treated with clinoptilolite, activated carbon and rock crystal.

4. A correlation between water parameters, wort fermentation dynamics and organoleptic evaluation of final drinks was established. The highest organoleptic rating was given to the drink samples prepared using water treated sequentially with clinoptil plate, activated carbon and rock crystal.

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