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THE EFFECT OF AERATION ON THE BIOCONVERSION OF CLITORIA TERNATEA L. TEA BY THE CULTURE OF MICROORGANISMS *MEDUSOMYCES GISEVII* DP-21

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Abstract

Traditional kombucha is produced by fermenting an infusion of black or green tea using a symbiotic culture of bacteria and yeast (SCOBY). According to the classification of microorganisms, this culture is called *Medusomyces gisevii*. It ferments sucrose and other components of the wort, which can serve as the basis for creating new products with original sensory properties beneficial to human health. The article determines the optimal temperature for extracting *Clitoria ternatea* L. tea leaves for the preparation of kombucha fermented beverage. It was established that the change in the color of the wort during fermentation is caused by an increase in its acidity and a corresponding change in the coloration of the fermented wort. The influence of aeration during fermentation on the appearance of the drinks was analyzed. The effect of dry matter content, particularly sucrose, on the fermentation process was studied. Changes in titratable acidity during the fermentation of wort under different cultivation conditions were determined. The content of acetic, gluconic, glucuronic, pyruvic, malic, citric, and succinic acids in the fermented wort was identified. The organoleptic profile of the drinks was examined, which confirms the feasibility of aerating the wort during fermentation.

Keywords: fermented beverages; kombucha; aeration; fermentation; physicochemical indicators; organoleptic indicators.

ВПЛИВ АЕРУВАННЯ НА БІОКОНВЕРСІЮ ЧАЮ *CLITORIA TERNATEA* L. КУЛЬТУРОЮ МІКРООРГАНІЗМІВ *MEDUSOMYCES GISEVII* DP-21

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Анотація

Традиційна комбуча виробляється шляхом ферментації настою чорного або зеленого чаю з використанням симбіотичної культури бактерій і дріжджів (SCOBY). За класифікацією мікроорганізмів ця культура має назву *Medusomyces gisevii*. Вона ферментує сахарозу й інші складові суслу, що може бути основою для створення нових продуктів із оригінальними сенсорними та корисними для здоров'я людини властивостями. В статті визначена оптимальна температура екстрагування листя чаю *Clitoria ternatea* L. для приготування ферментованого напою комбуча. Визначено, що зміна кольору суслу під час бродіння пояснюється підвищенням його кислотності і відповідною зміною кольорності збродженого суслу. Проаналізований вплив аерування під час бродіння на зовнішній вигляд комбучі. Досліджений вплив на процес бродіння вмісту сухих речовин, зокрема сахарози. Визначена зміна титрованої кислотності у процесі збродження суслу при різних умовах культивування. Визначений вміст у збродженому суслі оцтової, глюконової, глюкуронової, піровиноградної, яблучної, лимонної та янтарної кислот. Досліджений органолептичний профіль напоїв, що підтверджує доцільність аерування суслу під час збродження.

Ключові слова: ферментовані напої; комбуча; аерування; збродження; фізико-хімічні показники; органолептичні показники.

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Introduction

One of the current challenges is ensuring a high level of public health in Ukraine. The World Food Programme (WFP) foresees a significant increase in demand for functional food products and the necessity of expanding their production. The most pressing problems are malnutrition or overnutrition and imbalances in food consumption, which directly affect human health [1; 2].

The consumption of soft drinks, primarily sugary carbonated beverages, is one of the causes of obesity and related health problems [3]. An alternative is fermented beverages, which are gaining popularity worldwide [4]. Consumer demand for these beverages is a driving force in the growth of functional food production [5].

Although fermented products, including beverages, have been an important part of world culture for millennia, kombucha has only recently gained global popularity. This beverage began to spread in northeastern China in the II century BC and was used in Japan in the IV century AD as a medicinal remedy. Over time, it spread to European countries through trade routes [6].

It is known that kombucha is prepared by fermenting an aqueous extract of tea and sucrose using associations of bacteria and yeasts [7]. Among bacteria, the main representatives are *Acetobacter* and *Gluconobacter*. Yeasts are represented by numerous genera and species *Zygosaccharomyces*, *Candida*, *Kloeckera*/*Hanseniaspora*, *Torulaspora*, *Pichia*, *Brettanomyces*/*Cekkera*, *Saccharomyces*, *Lachancea*, *Saccharomycodes*, *Schizosaccharomyces* i *Kluyveromyces*) [8].

The popularity of kombucha has grown thanks to its high organoleptic qualities and beneficial properties. Its consumption is associated with health-promoting effects, including antimicrobial activity against pathogenic bacteria *Salmonella sp.*, *Escherichia coli*, *Staphylococcus aureus* i *Listeria monocytogenes in vitro*, anticancer properties against human colon and breast cancer, anti-inflammatory effects on human monocytic THP-1 cells, antioxidant activity, antihyperglycemic effects, and more [9–12]. These properties are linked to its composition, particularly organic acids (gluconic, glucuronic, malic, tartaric, pyruvic, citric, lactic, etc.), carbohydrates (fructose, glucose, sucrose, etc.), vitamins (B₁, B₂, B₆, B₁₂, C, etc.), biogenic amines and amino acids, purines, lipids, hydrolytic enzymes, ethanol, minerals, and carbon dioxide [13].

Traditionally, black and green tea are used as substrates for fermentation, but recently

manufacturers have employed alternative raw materials, such as fruits, vegetables, and herbs. Substrates of different origins affect the physicochemical properties, sensory characteristics, and functional features of kombucha [14]. At the same time, the components of raw materials may be metabolized into various biologically active compounds under the action of yeasts and bacteria.

Butterfly pea tea (*Clitoria ternatea L.*) is also known as «blue pea», «Darwin's pea», «Asian pigeonwings», or «shame flower». It is cultivated in subtropical regions, mainly in Southeast Asia, and is usually used as a natural food and beverage colorant. The flowers of this tea have a bright blue color due to anthocyanins, particularly the delphinidin glycoside [15], which has antidiabetic, antioxidant, antimicrobial, anti-inflammatory, and anticancer properties. This tea is used as a source of natural pigment with bioactive properties and can enhance the appearance and nutritional value of food products [16]. Anthocyanins, as flavonoids, are responsible for the red, blue, purple, and orange colors of vegetables, fruits, flowers, and leaves. They may be acylated with phenolic acids (p-coumaric, caffeic, ferulic, sinapic, gallic acids) and/or organic acids (malonic, acetic, malic, succinic, or oxalic), which can enhance the stability of food products. Acylation of anthocyanin molecules increases stability through intramolecular and/or intermolecular copigmentation and self-association reactions [17]. Research into the use of anthocyanin-containing extracts as a source of natural colorants in food products has increased in recent years due to the need to ensure consumer health.

In Asia, aqueous extracts of *C. ternatea* are traditionally used as a natural colorant in food and beverages. In Malaysia, it is used to color rice cakes and the popular dish Nasi Kerabu [18]. In Indian traditional and Ayurvedic medicine, it is used due to its strong therapeutic properties [19]. It has no cytotoxicity in human fibroblast cells and, accordingly, has a protective effect on human erythrocytes and inhibits plasmid DNA oxidation, confirming its toxicological safety and bioactivity. Moreover, *C. ternatea* may be a promising source of natural blue coloring with bioactive properties as an alternative to synthetic dyes. However, the stability of these pigments is influenced by factors such as pH, temperature, light, oxygen presence, solvents, presence of other pigments, metal ions, and enzymes [20].

The aim of this study was to determine the effect of aeration on the fermentation of wort by

Medusomyces gisevii DP-21 using *C. ternatea* tea leaves and sugar solution as raw materials.

To achieve this goal, technological modes of wort preparation and fermentation were defined, and the organoleptic profile of the finished beverage was studied.

The research adhered to the principles of food combinatorics, taking into account possible chemical interactions of the ingredients. Optimal methods of raw material introduction and processing were selected to ensure the efficiency of technological processes.

Materials and methods

The studies used drinking water in accordance with DSanPiN 2.2.4-171-10, *Clitoria ternatea* L. tea leaves according to regulatory documentation, sugar according to DSTU 4623-2006, and a deposited culture of microorganisms *Medusomyces gisevii* DP-21. The finished beverage was examined for compliance with the requirements of DSTU 4069:2016 «Soft Drinks. General Technical Conditions».

In raw materials, semi-finished products, and final beverages, physicochemical and organoleptic parameters were determined by standard methods. The mass fraction of soluble solids was determined refractometrically according to DSTU ISO 2173:2007; active acidity by potentiometric method according to DSTU 1132:2005; titratable acidity by volumetric titration according to DSTU 12147-2003.

The chemical composition of the initial and fermented wort was determined by chromatographic and spectrophotometric

methods. Sensory evaluation was conducted in accordance with DSTU ISO 6564:2005 «Sensory analysis. Methodology. Methods for establishing a flavor profile».

Samples of kombucha were prepared on the basis of wort obtained by adding an infusion of *Clitoria ternatea* L. tea (1%) to a sugar solution. The tea infusion was prepared by steeping tea leaves at 40...100 °C for 5 minutes. The wort was fermented for 14 days at 25 °C. The fermentation process was completed when the acidity of the beverage reached 2.5–4.5 cm³ of NaOH solution (1 mol/dm³) per 100 cm³ of beverage.

Organoleptic properties of the finished drink were evaluated by appearance, color, taste, aroma, and carbon dioxide saturation.

Results and discussion

During the fermentation of the wort with *Medusomyces gisevii* cultures, bioconversion of carbohydrates and other components of the wort took place. Newly formed compounds, together with the components present in the initial wort, led to the formation of biologically active substances, providing the beverage with a specific taste and aroma. The chemical composition of the wort changed significantly as a result of the culture's activity and depended on the substrate used.

The wort was prepared using *Clitoria ternatea* L. tea leaves and treated water. Extraction temperature, appearance, aroma, and the content of phenolic compounds and anthocyanins were determined. The results obtained are presented in Table 1.

Table 1

Effect of extraction temperature on organoleptic indicators and the content of phenolic compounds and anthocyanins in the extract

Criterion	Extract characteristics at temperature			
	40 °C	60 °C	80 °C	100 °C
Appearance	+	+	+	+
Aroma	-	+	+	+/-
Phenolic compounds	-	+	+	+/-
Anthocyanins	-	+	+	+

Note: «+» – positive effect; «-» – negative effect; «+/-» – effect not defined.

The extraction temperature is important for preserving biologically active substances, particularly anthocyanins, which are sensitive to high temperatures. Selecting the extraction temperature to achieve the maximum possible anthocyanin content is critical.

It was established that the extraction temperature influenced the studied parameters. The optimal temperature range was determined to be 60–80 °C. At these temperatures, anthocyanin losses amounted to 37–82 %.

It is known that kombucha has antioxidant, anticancer, anti-inflammatory, antidiabetic, and antibacterial properties. This beverage helps restore cell structure, which positively affects human well-being. It tones the body, provides energy, relieves fatigue, headaches, and nervous tension, and has probiotic effects. The drink contains carbohydrates, organic acids (including acetic, gluconic, oxalic, citric, malic, lactic, pyruvic, and ascorbic acids), and B-group vitamins.

Figure 1 presents a schematic diagram of *Medusomyces gisevii* metabolism, illustrating the

inversion of sucrose into glucose and fructose, followed by cellulose synthesis, its oxidation to gluconic acid, and primarily fermentation into ethanol, which is then oxidized by bacteria into acetic acid. It is clear that *Medusomyces gisevii* culture contains yeasts of the genus *Saccharomyces* and bacteria of the genus *Acetobacter*.

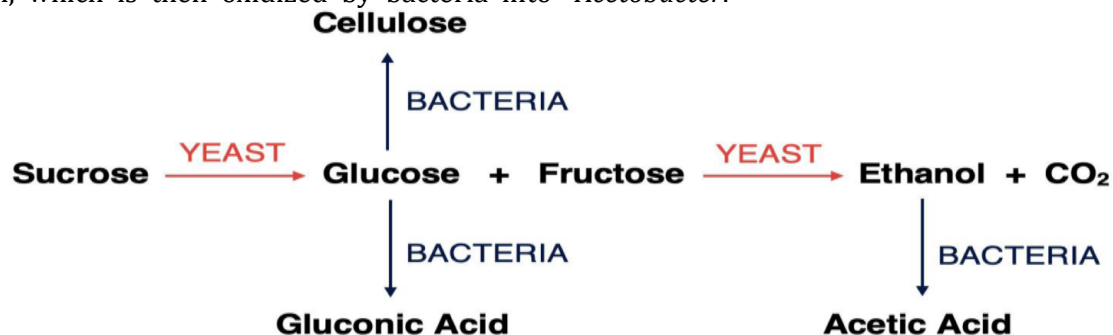


Fig. 1. Metabolic pathway of *Medusomyces gisevii*

The culture *Medusomyces gisevii* contains yeasts of different species and bacteria, which determine the formation of metabolic products and biomass in the form of a microbial film. At the beginning of fermentation, yeast invertase catalyzes the hydrolysis of sucrose into glucose and fructose, followed by alcoholic fermentation into ethanol, carbon dioxide, and secondary fermentation by-products. Alcoholic fermentation is accompanied by lactic acid fermentation of sugars. Acetic acid bacteria convert the formed ethanol into acetic acid. During fermentation, other enzymatic processes also occur, caused by the activity of other microorganisms that are part of the *Medusomyces gisevii* culture.

As a result of combined alcoholic, lactic, acetic, and other polyenzymatic processes, a fermented kombucha beverage is formed [7; 13].

Oxygen plays an important role in enzymatic processes during the cultivation of *Medusomyces gisevii*. The oxygen content in the wort and during fermentation affects the composition of the finished beverage and its flavor-aromatic properties. Figure 2 shows the appearance of wort during fermentation by *Medusomyces gisevii* DP-21 using *Clitoria ternatea* L. tea of different color varieties with aeration during fermentation (sample 2) and without free oxygen access (sample 1).

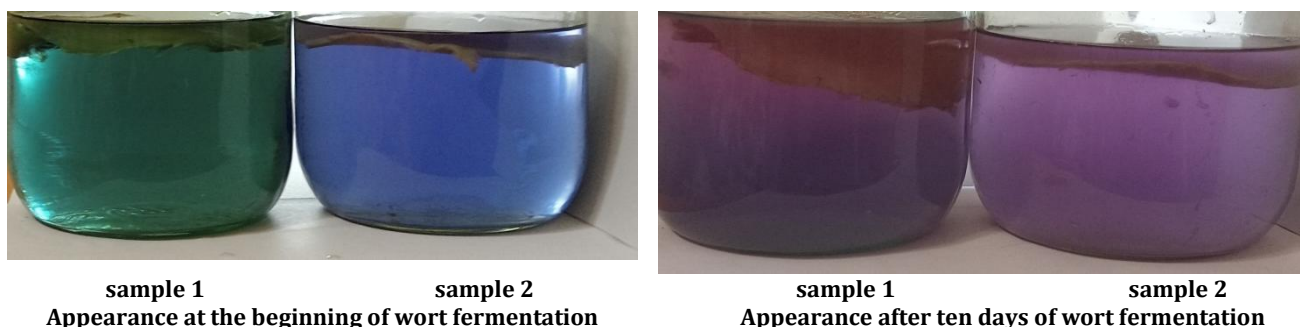


Fig. 2. Effect of aeration on the appearance of the beverage

According to the results of the conducted studies, it was established that during fermentation, the color of the wort changed. This is explained by an increase in acidity and, as a consequence, by a change in the color of the tea's pigment components. Aeration during fermentation had a corresponding effect on the appearance of the beverages, which is explained by the dynamics of changes in medium acidity during fermentation.

The dynamics of dry matter content in the wort of the studied samples were investigated over 14 days using the *Medusomyces gisevii* DP-21 culture.

It was established that during fermentation, the content of dry matter, particularly sucrose, decreased in all samples, while the content of glucose and fructose increased, which is explained by the activity of yeast invertase. The accumulation of simple sugars, glucose and fructose, occurred proportionally to the reduction in sucrose content. During fermentation, glucose and fructose were consumed, although their levels remained relatively stable. After the tenth day of fermentation, an increase in fructose content was observed in sample 1. More intensive fermentation of the wort was recorded in sample 2.

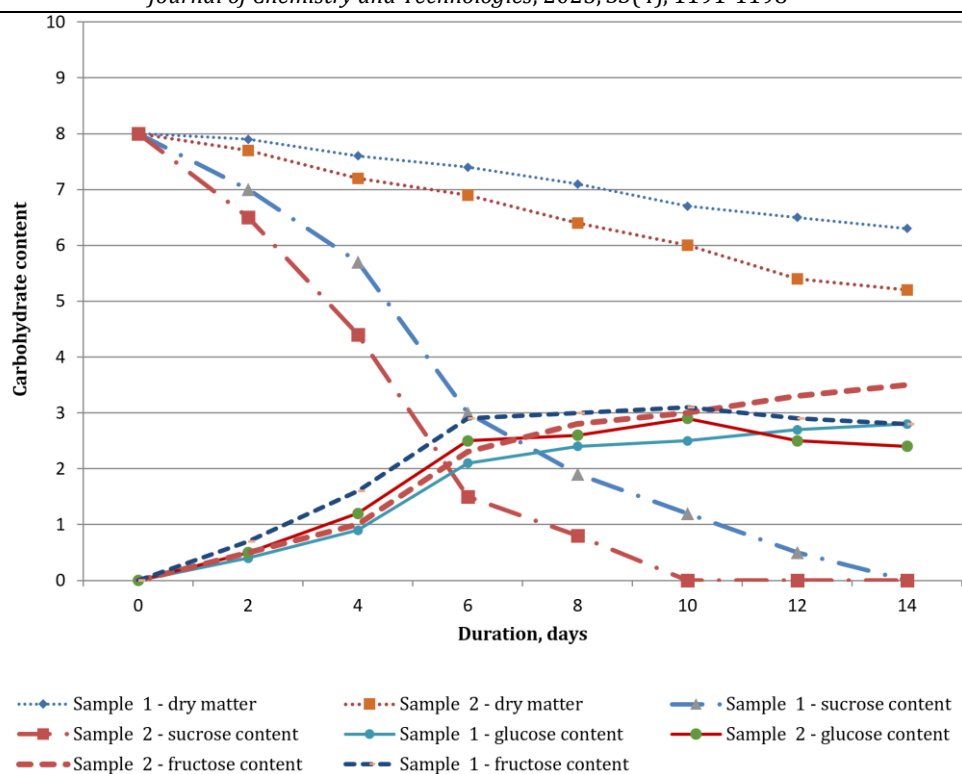


Fig. 3. Dynamics of dry matter and carbohydrate content during wort fermentation

It was established that during fermentation, the content of dry matter, particularly sucrose, decreased in all samples, while the content of glucose and fructose increased, which is explained by the activity of yeast invertase. The accumulation of simple sugars, glucose and fructose, occurred proportionally to the reduction in sucrose content. During fermentation, glucose

and fructose were consumed, although their levels remained relatively stable. After the tenth day of fermentation, an increase in fructose content was observed in sample 1. More intensive fermentation of the wort was recorded in sample 2.

Figure 4 shows the dynamics of titratable acidity for the studied samples.

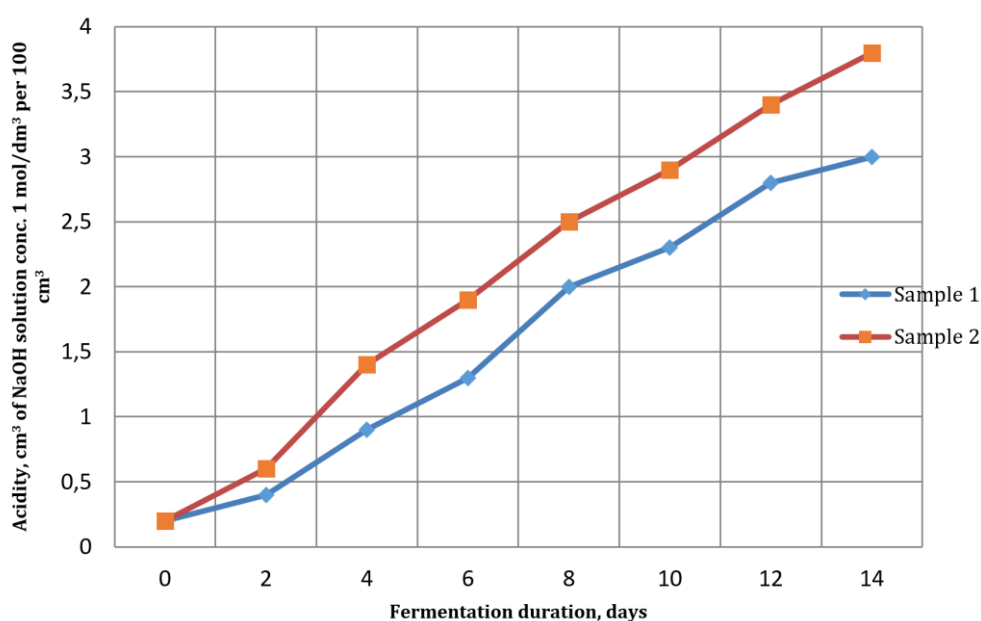


Fig. 4. Dynamics of titratable acidity during wort fermentation

During fermentation, titratable acidity of the wort increased 7–9 times. From the second day of fermentation, the intensity of acid formation in the aerated sample significantly differed from the non-

aerated sample. By the 14th day of fermentation, the acidity of sample 2 exceeded that of control sample 1 by 20 %.

The content of organic acids in the fermented wort samples is presented in table 2.

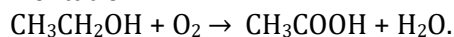
Table 2

Content of organic acids in fermented wort			
Organic acid content, mg/100 cm ³	Content of Organic Acids in Wort		
	at the beginning of fermentation	after fermentation	
		sample 1	sample 2
Acetic	0	1.32	1.76
Gluconic	0	1.01	1.24
Glucuronic	0	0.02	0.04
Pyruvic	0	0.6	0.9
Lactic	0	0.5	0.7
Malic	0.110	0.7	0.8
Citric	0.035	0.2	0.2
Succinic	0.004	0.03	0.04

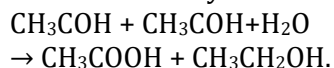
It was established that compared with the initial wort, the fermented wort contained acetic, gluconic, glucuronic, and pyruvic acids. A significant increase in malic, citric, and succinic acid content was explained by the activity of yeasts and bacteria in the *Medusomyces gisevii* DP-21 microbial consortium.

The accumulation of organic acids during fermentation was observed. Considering the metabolic relationships between yeasts and acetic acid bacteria of the *Medusomyces gisevii* consortium, the formation of organic acids occurs as follows.

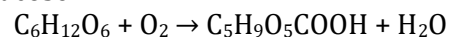
The highest proportion of fermented wort belongs to acetic acid, which is mainly produced by acetic acid bacteria from ethanol under the action of alcohol oxidase through acetic acid fermentation:



It is also possible for acetic acid to be produced by yeasts through the dismutation of acetaldehyde formed from pyruvic acid under the action of pyruvate decarboxylase:

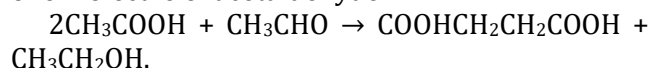


Gluconic acid is formed by the oxidation of glucose:



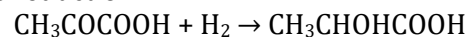
Oxidation of the aldehyde group of glucose leads to the formation of monobasic D-gluconic acid; oxidation of the primary alcohol group results in glucuronic acid; oxidation of both aldehyde and alcohol groups produces dibasic saccharic (glucaric) acid.

Succinic acid is formed by dehydrogenation and condensation of two molecules of acetic acid with one molecule of acetaldehyde:



The formation of succinic acid is also possible by deamination of glutamic acid.

Lactic acid may be formed from pyruvic acid by its reduction:



or by hydrolysis of phosphoglyceraldehyde.

Citric acid may be formed from acetaldehyde:

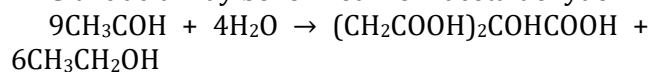


Figure 5 shows the organoleptic profile of the studied beverage samples at ratios optimal according to organoleptic indicators

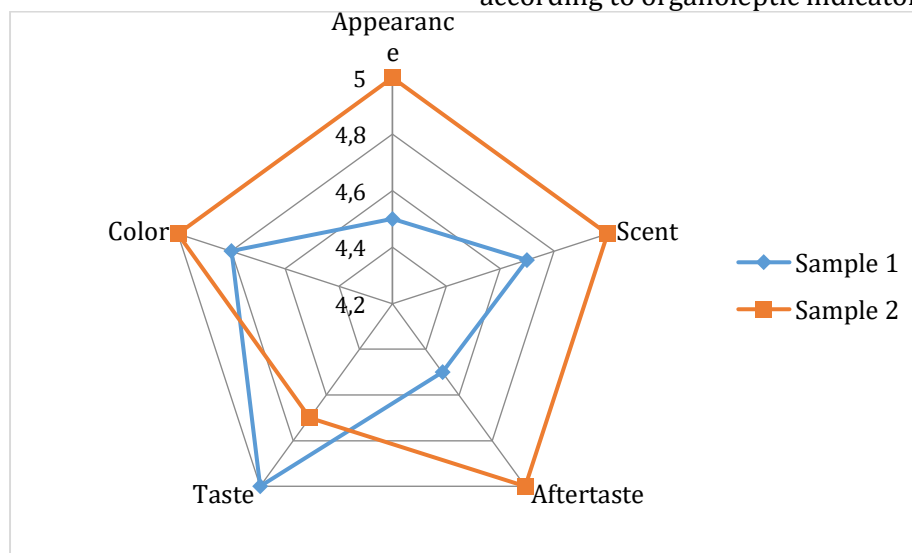


Fig. 5. Organoleptic profile of the beverages

It was established that sample 2 had the best scores for appearance, aroma, and aftertaste.

Thus, according to organoleptic parameters, the use of aeration during fermentation ensures the production of a beverage with high sensory qualities.

Conclusions

1. It was determined that the optimal extraction temperature of *Clitoria ternatea* L. tea leaves for the preparation of fermented kombucha beverage is 60–80 °C.

2. The change in wort color during fermentation is explained by an increase in its acidity and the corresponding change in the coloration of the fermented wort. Aeration during

fermentation does not have a significant effect on the appearance of the beverages.

3. During the fermentation of wort, the content of dry matter, particularly sucrose, decreases. By the end of fermentation, the fructose content in the wort increases.

4. Over 14 days of fermentation, the titratable acidity of the wort increases 7–9 times. Aeration during fermentation increases the acidity of the wort by 20 %.

5. The content of acetic, gluconic, glucuronic, pyruvic, malic, citric, and succinic acids in the fermented wort was determined.

6. The organoleptic profile of the beverages was identified, confirming the feasibility of aerating the wort during fermentation.

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