In high-gravity brewing, conditions arise that adversely affect the yeast. These are high osmotic pressure, high fermentation temperature, high ethanol content. As a result of these unfavorable factors, the intensity of yeast reproduction and the rate of fermentation decrease, the duration of the process increases, the degree of fermentation decreases, which leads to a change in the taste and aroma profile of the drink. Cofactors of important enzymes and stress modulators are microelement ions, the optimal content of which can be provided by adding appropriate salts to the wort.

In this work, the influence of calcium and zinc on the fermentation of high-gravity beer wort with the participation of Saccharomyces cerevisiae yeast strain Saflager W-34/70 was investigated.

The optimal dosage of $CaCl_2$ and $ZnSO_4$ salts was determined, which is 5.0 and 0.1 mg/dm^3, respectively. It was established that adding $CaCl_2$ to the wort leads to an increase in the rate of fermentation by 21.9 %, the apparent and actual degree of fermentation by 17.8 and 17.0 %, respectively. At the same time, the ethanol content in young beer increases by 19.1 %, the content of visible and actual extract decreases by 28.8 and 17.5 %, respectively, and the biomass of yeast accumulated during fermentation increases by 14.0 %. When using $ZnSO_4$, the changes in the values of all these indicators are significantly smaller and lie in the range of 3.2–5.8 %. Other physical-chemical parameters of the studied samples of young beer, namely acidity, pH value, content of vicinal diketones, do not undergo significant changes.

To enhance the growth and metabolism of yeast under adverse conditions that occur during high-gravity brewing, it is recommended to add $CaCl_2$ to beer wort in the amount of 5.0 mg/dm^3. This will make it possible to reduce the duration of fermentation of wort with a dry matter content of 18 % at a temperature of 15 °C by 1.5 days (21 %)

Keywords: high-gravity brewing, fermentation, yeast, microelement ions, calcium, zinc

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INTENSIFICATION OF HIGH-GRAVITY BREWER'S WORT FERMENTATION PROCESS WITH THE USE OF MICROELEMENTS

Ruslana Kosiv
PhD, Associate Professor
Department of Organic Products Technology
Lviv Polytechnic National University
Bandera str., 12, Lviv, Ukraine, 79013
E-mail: ruslana.b.kosiv@lpnu.ua

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1. Introduction

Brewing is one of the progressive sectors in the food industry, which is developing dynamically. In modern beer production, an important task has been to intensify the main fermentation process of beer wort, in particular high-gravity beer wort, while maintaining or improving the quality of finished products.

When processing highly extractable unsweetened or low-quality raw materials in wort, the content of components important for the yeast cell decreases: vitamins, amino nitrogen, macro- and microelements. This causes a decrease in the intensity of yeast reproduction, a slowing down of fermentation and an increase in its duration, a decrease in the degree of fermentation, changes in the taste and aroma of the drink.

Therefore, it is a relevant task to carry out studies on devising ways to stimulate the growth and metabolism of yeast under the conditions of brewing, in particular, high-gravity brewing.

2. Literature review and problem statement

In high-gravity brewing, conditions arise that adversely affect the yeast. These include high osmotic pressure caused by an increased concentration of dry substances in the wort, increased temperature at the main fermentation stage, and increased ethanol content. Under the influence of these factors, the productivity of yeast decreases, especially when the initial concentration of wort is more than 18 % by mass. This often leads to slow and incomplete fermentation and undesirable changes in the taste and aroma profile of the resulting beer [1]. The degree of these changes depends on the concentration of the initial wort and the fermentation temperature.

Better quality beer is obtained during cold fermentation. The content of substances that form the bouquet of finished beer is lower than the threshold of sensitivity due to the fact that metabolic reactions proceed more slowly and not so deeply [2]. However, at such a temperature, the fermentation activity of yeast is low, and therefore the duration of the main fermentation stage of high-gravity wort is longer than with traditional technology, which is economically impractical.

In order to intensify the technological processes of brewing beer, a warm technique is used. Elevated temperatures generally contribute to the intensification of fermentation but lead to beer with an inharmonious taste because the content of certain taste-aromatic substances, in particular acetaldehyde, ethyl acetate, and 2-methyl-butanol, exceeds the threshold of perception [2, 3].

Nutrient and growth substances are added to the wort to prevent a decrease in the intensity of reproduction and fermentation activity of yeast. The main mineral components needed for the growth and reproduction of yeast include N, P, K, S, and Mg, and microelements include Ca, Mn, Fe, Co, Cu, and Zn. When choosing preparations that contain these substances, and to determine their dosage, they take into account the yeast's need for growth factors and mineral components. Thus, the need for trace elements can increase several times if the yeast is under the influence of stress factors. Such factors include, in particular, an increased content of dry substances or ethanol in the medium and a fermentation temperature higher than the optimal value.

Among trace elements, the influence of zinc on the fermentation activity of yeast has been studied to the greatest extent. It has been established that it affects the efficiency of fermentation in brewing and winemaking [4]. The recommended concentration of this trace element in wort is more than 0.15 mg/dm^3. If its concentration is insufficient, problems arise at the fermentation stage and, as a result, the quality of beer deteriorates [5]. In view of this, the presence of an appropriate amount of zinc in the environment is considered a necessary condition for ensuring the optimal rate of fermentation of brewer's yeast [6]. Among zinc salts – sulfate, chloride, and nitrate – the highest absorption of the trace element was observed when $ZnSO_4$ was added to the wort. It was found that the introduction of various sources of zinc at a concentration of 3 mg/dm^3 led to an increase in the yield of yeast biomass in the range of 1.00–1.03 g per 100 cm^3 of the culture medium. However, a higher concentration of the trace element caused a decrease in the yield of yeast biomass [7]. Adding zinc nanoaquachelate with a zinc concentration of 0.10 mg/dm^3 increased the fermentation activity of brewer's yeast, and the duration of the main fermentation process of beer wort with a concentration of 11 % by mass decreased by 1–2 days [8]. Despite numerous works that study the influence of zinc on the fermentation of beer wort, the question of the role of this cation in the fermentation of a substrate with a high content of fermentable sugars, in particular, high-gravity beer wort, remains unresolved.

Unlike zinc, the effect of calcium level on beer wort fermentation has not been studied much. The authors of paper [9] established that the addition of $CaCl_2$ in amounts of 1 and 10 mg/dm^3 significantly improves the ability of yeast to grow at high glucose concentrations. However, as is known, beer wort contains a small amount of glucose (up to 12 %). Instead, it is dominated by maltose disaccharide, the content of which is approximately 65 %.

All this gives reason to assert that it is appropriate to conduct a study on determining the stimulating effect of calcium and zinc on the growth and metabolism of yeast under the conditions of high-gravity brewing.

3. The aim and objectives of the study

The aim of this work is to improve the fermentation process of high-gravity beer wort with the use of microelements, which will make it possible to avoid slow and incomplete fermentation, undesirable changes in the main indicators of the obtained beer, and to reduce the duration of the process.

To achieve the set goal, the following research tasks must be solved:

– to determine the dosage of salts of microelements under the conditions of high-gravity brewing;

– to investigate the influence of microelement salts on the dynamics of the main fermentation and the degree of fermentation of young beer;

– to investigate the effect of salts of trace elements on the main physical-chemical parameters of young beer and the biomass of accumulated yeast.

4. The study materials and methods

The object of research is the fermentation process of high-gravity beer wort using salts of trace elements.

The subject of research is high-gravity beer wort, yeast, young beer.

Based on the available knowledge and experience, it can be assumed that microelements Ca and Zn will have a stimulating effect on the fermentation process of high-gravity beer wort. With this in mind, the influence of their water-soluble salts $CaCl_2$ and $ZnSO_4$ on the course of fermentation of wort with a concentration of 18 % dry matter and on the main indicators of young beer was studied. The control was wort without additional introduction of the sources of the studied microelements.

The wort was fermented with the participation of bottom-fermenting yeast *Saccharomyces cerevisiae* strain Saflager W-34/70. Yeast was cultivated in three stages in beer hopped sterile wort with a dry matter concentration of 12 %. At the first stage, 10 cm^3 of wort was introduced into a test tube and pure yeast culture was sown. At the second stage, 50 cm^3 of wort was added to the flask and the yeast from the first stage was seeded. At the third stage, 200 cm^3 of wort and yeast from the second stage were added to the flask. At all stages, yeast was cultivated for 24 hours at a temperature of 25 °C. The obtained yeast biomass was separated from the culture medium by centrifugation for 10 min at a rotation frequency of 4000 min^{-1}.

Fermentation of 200 cm^3 of wort was carried out periodically for 7 days at a temperature of 15 °C, which is optimal for the used strain. The initial concentration of yeast was 30 million cells/cm^3. During fermentation, the mass of released CO_2 (g) was controlled by decreasing the mass of the fermentation plant. After the completion of fermentation, the yeast was separated from the young beer by centrifugation for 10 min at a speed of 4000 min^{-1}.

I determined the physical-chemical parameters of the obtained samples of young beer according to the methods of analysis accepted in the industry:

– concentration of wort (% wt.) and apparent extract of beer (% wt.) – using the hydrometric method (MEBAK. B-590.09.900. Apparent extract);

– ethanol content (% wt.) and actual beer extract (% wt.) – pycnometrically after beer distillation (MEBAK. B-590.10.024. Original wort, extract, and alcohol – by distillation (official method));

– pH of beer – potentiometrically (MEBAK. B-590.00.040. pH measurement);

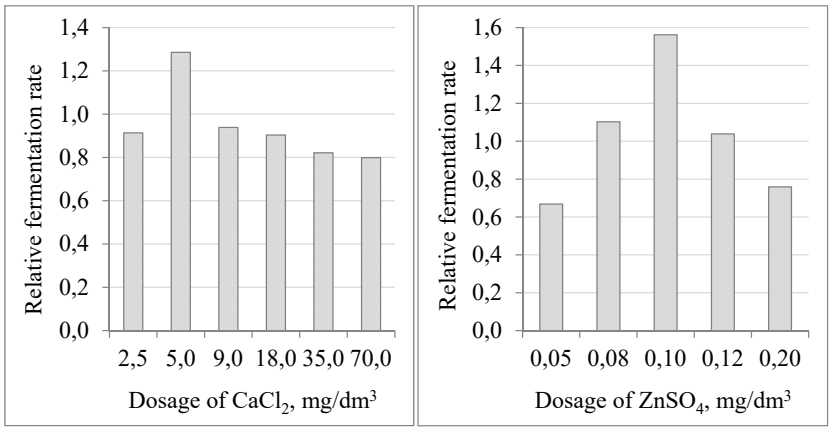
– the content of vicinal diketones in beer (mg/dm^3) – by the spectrophotometric method (MEBAK. B-420.21.111 Vicinal Diketones – Photometric Method).

Biomass of yeast separated by centrifugation (g) was determined by weighing.

5. Results of investigating the effect of salts of trace elements on the course of biochemical processes of fermentation of high-gravity beer wort

5. 1. Determining the dosage of salts of trace elements

First, the optimal dosage of salts of trace elements was determined: $CaCl_2$ – in the range from 2.5 to 70 mg/dm^3 of wort, $ZnSO_4$ – in the range from 0.05 to 0.2 mg/dm^3. To this end, 50 cm^3 of 18 % wort was fermented with the addition of various amounts of salts for a short period (5.5 hours). Graphical dependences of the mass of carbon dioxide released as a result of fermentation on the duration of the process were constructed and the rate of fermentation was determined by the angle of inclination of the curves. The optimal dosing of salts was determined by the maximum speed of the process (Fig. 1).



a *b*

Fig. 1. Dependence of the fermentation rate (relative to the control) on the trace element salt dosage: *a* – $CaCl_2$; *b* – $ZnSO_4$

Based on the results, it was determined that the optimal dosage of $CaCl_2$ is 5.0 mg/dm³, and $ZnSO_4$ is 0.1 mg/dm³.

5. 2. Studying the influence of trace element salts on the dynamics of the main fermentation and the degree of fermentation of young beer

The effect of trace element salts on the course of biochemical fermentation processes and physicochemical parameters of young beer was studied at the determined optimal concentration. The results were compared with the control sample.

The dynamics of the main fermentation of high-gravity beer wort were studied by the mass of carbon dioxide released as a result of fermentation (Fig. 2). It was found that when $CaCl_2$ is used, the rate of fermentation increases significantly – by 21.9 %, and when $ZnSO_4$ is added – only by 4.3 %.

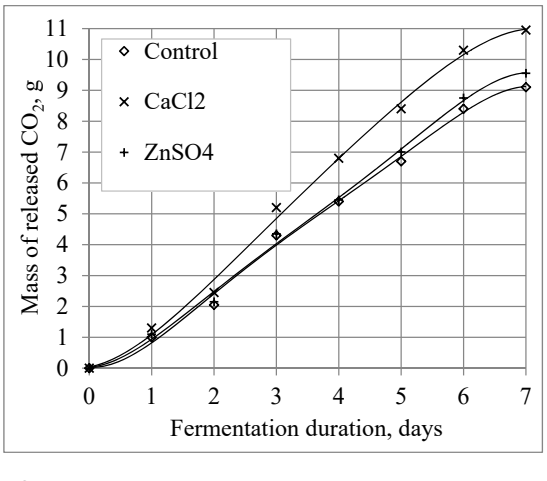


Fig. 2. The influence of microelement salts on the dynamics of the main fermentation of high-gravity beer wort

Based on the determined actual and apparent content of extractive substances in young beer and the content of extract in the initial wort, the actual and apparent degree of beer fermentation was calculated and the influence of trace element salts on these indicators was determined.

It was established that adding $CaCl_2$ to the wort leads to an increase in the apparent and actual degree of fermentation by 17.8 and 17.0 %, respectively (Fig. 3). On the other hand, when $ZnSO_4$ is added to the wort, the apparent and actual degree of fermentation increases by only 4.0 and 3.9 %, respectively.

The results (Fig. 2) demonstrated that due to the use of salts of trace elements, the duration of the main fermentation process of high-gravity beer wort can be shortened. Adding $CaCl_2$ to the wort in the amount of 5.0 mg/dm³ makes it possible to reduce the duration of the process by 1.5 days (21 %). In contrast, when using $ZnSO_4$, the fermentation process ends only 0.5 days (7 %) earlier than the control sample.

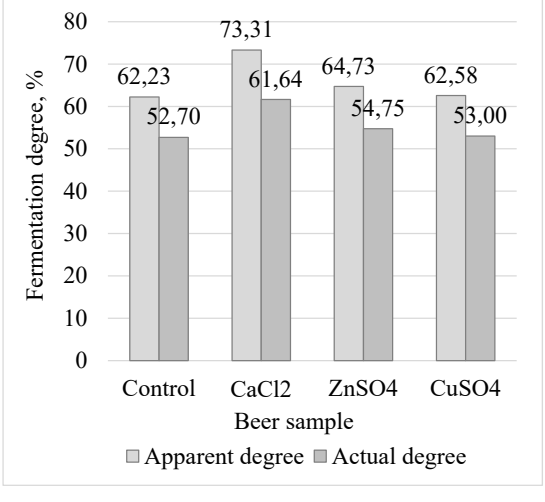


Fig. 3. Influence of salts of trace elements on the degree of fermentation of young beer

5. 3. Studying the effect of salts of trace elements on the main physicochemical parameters of young beer and the biomass of accumulated yeast

The results of investigating the effect of salts of trace elements on the physical and chemical parameters of young beer are given in Table 1.

Table 1

The effect of salts of trace elements on the physical and chemical parameters of young beer

Beer indicators	Beer samples		
	Control	$CaCl_2$, 5.0 mg/dm³	$ZnSO_4$, 0.1 mg/dm³
Visible extract content, % wt.	6.94	4.94	6.54
Actual extract content, % wt.	9.15	7.55	8.86
Ethanol content, % wt.	4.93	5.87	5.19
pH	4.458	4.367	4.432
Acidity, cm³ of NaOH solution with a concentration of 1 mol/dm³ per 100 cm³	3.7	3.3	3.5

It was found that under the influence of $CaCl_2$, due to faster and deeper fermentation, the content of visible and

actual extract in young beer decreases by 28.8 and 17.5 %, respectively. At the same time, the ethanol content increases by 19.1 %. The changes in the values of these indicators in the beer sample obtained using $ZnSO_4$ are not so significant and amount to 5.8, 3.2, and 5.3 %, respectively.

The pH value of all young beer samples is within acceptable limits – no more than 4.5. Their acidity ranges from 3.3 to 3.7 cm^3 of NaOH solution with a concentration of 1 mol/dm^3 per 100 cm^3, which meets the requirements of the state standard for finished beer obtained from 18 % wort.

Vicinal diketones – diacetyl (2,3-butanedione) and 2,3-pentanedione – play a significant role in shaping the taste of beer. When their concentration limits are exceeded, they give beer an unpleasant taste (from sweet to honey) and an unpleasant aroma. Given this, the task of brewers is to minimize their formation or speed up their recovery to substances with a higher threshold of sensation.

It was established that the content of vicinal diketones in beer obtained using $ZnSO_4$ is the same as in the control sample (Fig. 4). In the beer sample obtained using $CaCl_2$, the content of these diketones is higher than the control but lower than the limiting value of 0.3 mg/dm^3 established for young beer.

During the fermentation of beer, in addition to the formation of the main (alcohol and carbon dioxide), secondary and by-products, which largely determine the taste and aroma of the drink, there is an accumulation of yeast. At the same time, their biomass increases three to four times. The repeated use of such yeast in subsequent fermentations (serial repitching) contributes to cost savings compared to the use of yeast cultured for each fermentation. After all, the cultivation of yeast is a very time-consuming process, which consists of several stages and is associated with costs for sterilization and cooling of the wort [1].

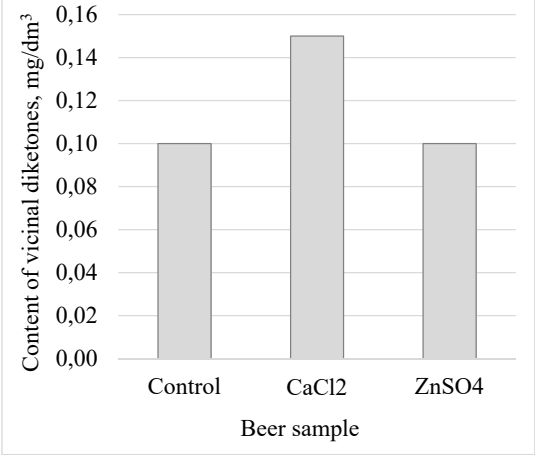


Fig. 4. The influence of trace element salts on the content of vicinal diketones in young beer

The addition of salts of microelements helps increase the biomass of yeast accumulated during fermentation (Fig. 5).

In young beer obtained using $CaCl_2$, 14.0 % more yeast accumulates than in the control sample. When using $ZnSO_4$, the increase in biomass is much smaller and amounts to 4.7 %.

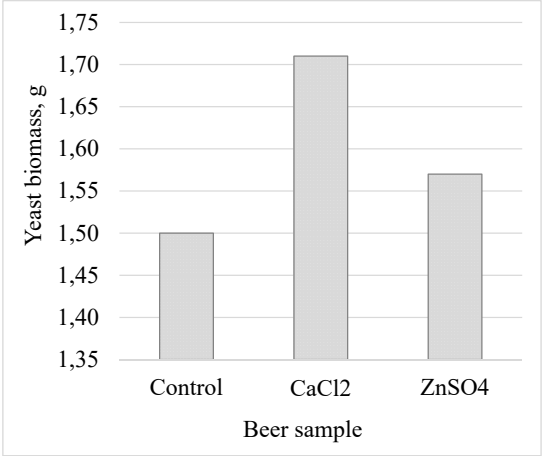


Fig. 5. Effect of trace element salts on yeast biomass accumulated during fermentation

6. Discussion of results of investigating the process of fermentation of high-gravity beer wort with the use of microelements

During the fermentation of highly concentrated beer wort, the yeast is subjected to physical and chemical stress. Physical stresses include elevated temperatures, pressure, and osmotic stress. Regarding chemical stresses, the most common are metabolites and/or metal ions, as well as nutrient depletion and pH.

On the basis of my research, it can be concluded that to ensure effective fermentation of high-gravity wort by yeast and to obtain beer of proper quality, it is necessary to adjust the composition of the wort according to the content of microelements. Optimum values of their content can be achieved by adding appropriate salts to the wort.

Micronutrients are important factors for yeast growth. Their presence in beer wort in small quantities has a beneficial effect on the vital activity of yeast cells. Microelements activate enzymes, increase fermentation activity, improve flocculation. However, higher concentrations can cause colloidal turbidity, and high levels of heavy metal ions are toxic to yeast.

In this study, it was established that Ca^{2+} ions have a significant stimulating effect on the fermentation of high-gravity beer wort. The results showed that, owing to the use of $CaCl_2$, the duration of the main fermentation stage can be shortened by 1.5 days (Fig. 2). Such results can be explained by the influence of calcium on the structure and functions of mitochondria in an environment with high osmotic pressure. It was established [9] that the addition of $CaCl_2$ in amounts of 1 and 10 mg/dm^3 significantly improves the ability of yeast to grow at high glucose concentrations. This is due to the effect on their physiological state, the enzymes of the tricarboxylic acid cycle and the cytochrome system. In addition, calcium ions play a key role in the important process for brewing – flocculation of yeast cells [10].

The stimulating effect of Ca^{2+} ions during the fermentation of beer wort was compared with the effect of Zn^{2+}, which has been studied to a greater extent. It was established that the use of $ZnSO_4$ makes it possible to reduce the duration of the main fermentation of high-gravity beer wort by only 0.5 days (Fig. 2).

The authors of work [8] reported a different result from the one described above. They investigated that with the addition of zinc nanoaquachelate (zinc concentration of 0.10 mg/dm^3), the duration of fermentation of beer wort with a concentration of 11 % by mass decreases by 1–2 days. Such a difference is probably related to the fact that substrates with different dry matter content – 11 and 18 % – were fermented, and zinc was used in different forms.

The stimulating effect of zinc can be explained by the fact that it is an important trace element for yeast, which affects the growth and metabolism of cells. If there is a deficiency of zinc in beer wort, this leads to a decrease in the rate of fermentation, so-called sluggish fermentation occurs. This phenomenon depends on the yeast strain and can occur when the zinc level in the wort is lower than 0.1 ppm. Zinc plays an important role in the enzymatic metabolism of yeast not only because it is necessary for the activity of ethanol dehydrogenase – the final Zn-metalloenzyme in the chemistry of alcoholic fermentation. Zinc is also needed because it can stimulate the absorption of maltose and malt triose by brewer's yeast cells, which leads to an increase in the rate of fermentation [10]. Zinc acts as a cell membrane stabilizer, plays a crucial role in gene expression and genome modification, and activates almost 300 enzymes [4]. It was established [11] that increasing the concentration of zinc had a positive effect on the content of trehalose in yeast cells. Trehalose, together with other reserve substances, determines the physiological state of yeast, directly affects the course of fermentation, participates in maintaining the viability of cells in stressful conditions.

It was established that the pH value and acidity of young beer samples obtained by fermentation of wort with separate introduction of $CaCl_2$ and $ZnSO_4$ differ slightly from the control and correspond to the norms for finished beer (Table 1). The content of vicinal diketones in the beer sample with $ZnSO_4$ is similar to that in the control, and in the sample with $CaCl_2$ it is slightly higher than the control but within the acceptable limits for young beer.

According to the results of my research, it was found that the addition of calcium and zinc salts, despite the acceleration of the main fermentation, leads to an increase in the biomass of accumulated yeast (Fig. 5). The positive effect of Zn^{2+} cations on the yield of yeast biomass was also observed by the authors of paper [7].

So, the question of the possibility of using salts of trace elements to eliminate the disadvantages of high-gravity wort fermentation and shorten the duration of the main fermentation stage has been resolved.

However, the current research has its limitations because the effect of introducing salts of trace elements can be achieved under certain conditions. In particular, it will depend on the duration and storage conditions of seed yeast, their physiological state. This effect will also be influenced by the composition of the wort (especially the content of amino nitrogen), the number of generations of seed yeast, the method of main fermentation, the type of beer (light, semi-dark, dark). With an increase in the number of generations, the physiological condition of yeast deteriorates due to the adsorption of protein-tannin complexes, bitter hop substances and bacteria that infect beer on their surface. In this case, it is advisable to increase the consumption of salts. However, in the presence of a significant number of foreign microorganisms, the number of additives should be reduced, or their use should be abandoned since at the same time as the intensification of yeast growth and wort fermentation, the multiplication of foreign microorganisms will occur.

In-depth research on the influence of Ca^{2+} and Zn^{2+} ions on the content of taste-aromatic substances in beer, in particular secondary fermentation products, is needed. The authors of work [4] established that the enrichment of yeast cells with zinc by means of "preconditioning" affects the increase in the number of flavor congeners in distillates obtained from fermented media. Such distillates are characterized by a changed profile of taste and aroma. Zinc preconditioning of yeast cells increased the production of some higher alcohols, although this was primarily related to the yeast strain.

It would also be advisable to investigate the effect of the simultaneous addition of salts of various cations on the course of the main fermentation of high-gravity wort and the parameters of the resulting beer.

7. Conclusions

1. The optimal dosage of salts of microelements for the intensification of fermentation of high-gravity beer wort was determined. The optimal dosage of $CaCl_2$ is 5.0 mg/dm^3, and $ZnSO_4$ is 0.1 mg/dm^3.

2. The influence of microelement salts on the dynamics of the main fermentation and the degree of fermentation of young beer was studied. Adding $CaCl_2$ to the wort leads to an increase in the rate of fermentation by 21.9 %, and the apparent and actual degree of fermentation by 17.8 and 17.0 %, respectively. The effect of using $ZnSO_4$ is much smaller: the changes in the values of these indicators are 4.3, 4.0, and 3.9 %, respectively.

3. The effect of microelement salts on the main physicochemical parameters of young beer and biomass of accumulated yeast was investigated. Addition of $CaCl_2$ to the wort leads to a decrease in the content of visible and actual extract in young beer by 28.8 and 17.5 %, respectively. At the same time, the ethanol content increases by 19.1 %, and the biomass of yeast accumulated during fermentation by 14.0 %. Changes in the values of all these indicators when using $ZnSO_4$ are much smaller and lie in the range of 3.2–5.8 %. To enhance the growth and metabolism of yeast under adverse conditions that occur during high-gravity brewing, it is recommended to add $CaCl_2$ to beer wort in the amount of 5.0 mg/dm^3. This will make it possible to reduce the duration of fermentation of wort with a dry matter content of 18 % at a temperature of 15 °C by 1.5 days (21 %).

Conflicts of interest

The author declares that she has no conflicts of interest in relation to the current study, including financial, personal, authorship, or any other, that could affect the study and the results reported in this paper.

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Data availability

All data are available in the main text of the manuscript.

Use of artificial intelligence

The author confirms that she did not use artificial intelligence technologies when creating the current work.

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