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ORIGINAL PAPER

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APPLICATION OF ULTRASOUND FOR ENHANCING FERMENTATION RATES IN BREWING TECHNOLOGY

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ABSTRACT

Background. There is much promise in technologies which may speed up time-consuming processes such as preparing seed yeast, primary fermentation and improving beer quality in the brewing industry. This study focuses on the activating and disintegrative effect of ultrasound with a 44 kHz frequency and a 1.0 W/cm^2 intensity on brewer's yeast.

Materials and methods. This study established that ten-minute ultrasonic treatment of yeast is sufficient to reach the stimulating effect. Further ultrasonic treatment is irrelevant since the percentage of dead cells in the yeast suspension exceeds the permissible levels (more than 10%). The experiment showed that two-minute ultrasonic treatment improved the physiological activity of seed yeast and shortened the time for producing seed yeast by 12 hours. Ultrasonic disintegration allowed a yeast extract to be obtained from the brewer's spent yeast. Ultrasound was applied to the yeast suspension for 19 minutes.

Results. The obtained yeast extract was used for additional nutrition in preparing seed yeast. It was found that the added yeast extract (2% of the total volume) shortened the time for preparing seed yeast by 6 hours due to the improved physiological state of the yeast. At the final stage, two-minute ultrasonic treatment and yeast extract (2% of the total volume) were used to activate the seed yeast.

Conclusion. The seed yeast activation shortened the time for preparing seed yeast by 18 hours, and for the primary fermentation by 24 hours, while also improving the quality of the beer.

Keywords: barley, malt, wort, ultrasound, yeast extract

INTRODUCTION

Improving the quality of finished products and shortening the time required for their manufacture are critical factors for increasing competitiveness among breweries (Bamforth, 2016; Kalugina et al., 2019).

Malt obtained from malting barley varieties is the primary raw material in brewing. The Mikhailovsky variety of barley has proved competitive in the Republic of Bashkortostan's soil type and climatic conditions. Over the years this barley has continuously demonstrated its high yield potential and brewing properties across the zones of the republic (Gusev and Kadikov, 2018). Barley is the main raw material for brewing; the quality of barley affects the malt obtained from the grain and the beer wort used in fermentation.

The seed yeast preparation and the primary fermentation take from 9 to 12 days, and the length of

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these processes is affected by the physiological state of the yeast (Bamforth, 2016). Various techniques have been employed to stimulate yeast growth. Currently, there are various methods for activating the yeast.

Physical methods require that electromagnetic radiation and acoustic vibrations are applied to the yeast cells (Karpenko and Chulanov, 2008).

Chemical and biochemical methods involve introducing chemical compounds and growth substances necessary to reproduce and develop yeast cells into the liquid culture (Karpenko and Chulanov, 2008).

The effect of ultrasound on food-related processes is studied extensively in Russia and other countries. In the food industry, ultrasound is used to assist extraction, crystallization, freezing, emulsifying, filtration and drying processes. The stimulating effect of ultrasound on microorganisms and enzymes is widely recognised. It has been found that the treatment of yeast with ultrasonic acoustic vibrations causes changes in the physiological state of cells. This process depends on the intensity and duration of the ultrasound treatment. Shestakov and Volokhova (2000) introduced a technique for activating pressed and dried baking yeast by ultrasound for yeast dough preparation purposes. Our previous studies focused on the effects of ultrasound on distiller's yeast.

Disposal and recycling of food processing waste is an urgent issue. Spent brewer's yeast is brewery waste whose amount is estimated to be thousands of tons. The spent brewer's yeast can be reused to produce yeast extracts, which are rich sources of amino acids, macro- and microelements, vitamins and other biologically active compounds (Jacob et al., 2019; Puligundla et al., 2020). Long ultrasonic treatment ensures yeast cell disruption and release of nutrients into the liquid culture (Bystryak et al., 2015).

Also, in terms of economic efficiency it is crucial to accelerate technological processes in brewing; this goal can be reached by reducing the fermentation time.

This study aimed to develop a method for accelerating the seed yeast preparation and primary fermentation processes with ultrasound and yeast extract.

The main objectives of this study are listed below:

- to prepare wort from malt (the Mikhailovsky barley variety) using conventional technology
- to study the activating and disintegrative effects of ultrasound on yeast cells
- to examine the morphological and biosynthetic characteristics of seed yeast activated by yeast extract
- to analyse the indices of light beer produced by the activated yeast.

MATERIALS AND METHODS

The experiment was carried out in Bashkir State Agrarian University and the central analytical laboratory of the Bashkir Research Institute of Agriculture, Ufa, Russia.

The objects of the study

The Mikhailovsky barley variety, grown in the Republic of Bashkortostan. The plant is medium-sized (50–77 cm). The grain is a large-sized, rhombic shape. The variety is mid-ripening and the growing season lasts 72–92 days.

The brewer's yeast is *Saccharomyces cerevisiae*, which is top-fermenting.

The hopped wort (12% original gravity) was used. It was produced at the Ufa Brewery LLC (OOO "Ufimsky Pivovarennyy Zavod") using the conventional technology.

The yeast suspension was activated by ultrasound and yeast extract in the cooled hopped wort (14°C) after pitching the seed yeast. The seed yeast was prepared by diluting the pure culture.

Ultrasound was provided in an ultrasonic bath which produced ultrasonic vibrations with a frequency of 44 kHz and an intensity of 1.0 W/cm^2. A UB--14/100-MP-22/44-kHz ultrasonic bath (Manufacturer: RELTEC, Russia) was used in the study. This bath is controlled by a microprocessor unit and has a 14-litre tank capacity.

Methods of the study

- Russian standard requirements (GOST) were used to assess the quality of malting barley and malt (Brewer's barley, 2010; Malt, 2016)
- a yeast counting instrument "SPARE PART LIST", Model 902 Yeast-2004 was used to calculate the total number of cells
- microscopic examination was used to assess the number of dead cells, glycogen-containing and budding cells

- the weight method was employed to determine the fermentative activity of yeast
- the organoleptic and physico-chemical parameters of the beer were checked for compliance with Russian standard requirements (GOST) (Beer, 2019)
- vitamins were determined using standard practices (Altria, 1998; DIN EN 15607 Foodstuffs, n.d.).

Statistical processing of the results was carried out using a software package product of StatSoft Inc. (Statistica 10.0). The tables and figures show the average values obtained from three biological repeats. The relative standard deviation of the results does not exceed 5%.

RESULTS AND DISCUSSION

Barley and malt characteristics

The chemical composition of barley used for brewing purposes must comply with several regulatory requirements. Therefore, the first stage of the study analysed barley and light malt obtained from grain. The results of the analysed barley were compared with the standard requirements (Brewer's barley, 2010). The study barley met the standard requirements in both organoleptic and physico-chemical parameters. It had a yellow colour, smelled as it should, and its humidity was below the critical value (14%). The protein content was 11.5%, the grain size was 88%, the germination capacity was 96%, and the viability was 97%. The indices are considered adequate for the Republic of Bashkortostan's climatic conditions since growing malting barley is not traditional in the region. The average thousand-grain weight was 57 g. The barley had 0.5 and 1.5% of weed seed and foreign grain, respectively. According to the Russian standard for malting barley, the study barley was of first-class quality.

Before preparing the wort at the Ufa brewery, the light malt physical and chemical parameters were examined and compared with the Russian standard requirements (Malt, 2016).

The malt was yellow and had a sweet malt aroma and flavour. The moisture content was 4.5%, whereas the standard value is 5%. The sieve analysis showed that the number of particles passing through the sieve (sized 2.2*20) was 3%. The extractivity of the malt was 83%, a valid quality indicator for the malt produced from high-protein barley.

The above-listed indices and the standard indicators (the Kolbach index, the number of farinaceous and vitreous kernels) meet the requirements established for light malt of first-class quality. The obtained malt was kept in the proper storage conditions at a relative humidity of 19%. This factor is crucial for barley of all classes. Storage of malt at a higher humidity adversely affects the malt processing and the final product quality. As a result, the extractivity and sugar content of malt decline and the microbial content rises. The hopped wort was then prepared. The produced wort was transparent on visual inspection. Saccharification lasted 17 minutes; the colour was 3.0 EBC units and the acidity was 1.0 unit. All of the indices meet the Russian standard requirements.

The effects of ultrasound on yeast

The next stage involved assessing the effects of ultrasound on seed yeast.

The wort was cooled to 14°C, and the seed yeast was pitched to achieve 20×10^6 per 1 cm^3 concentration. The yeast suspension was subjected to ultrasound for 35 minutes. The temperature was measured and the percentage of dead cells was assessed during the first 20 minutes and at 25, 30 and 35 minutes (Fig. 1).

The number of dead cells exceeded 10% from 5 min of the treatment; the index was higher than the acceptable production standards. It was also found that the treatment caused heating of the medium; the temperature reached 29°C by 20 min. The heating of the medium was due to the ultrasonic wave energy being converted into heat energy. The previous study has proved that activation of yeast suspension by ultrasound is not associated with the related thermal effect. In terms of fermentative activity, the yeast activated by ultrasound with the thermal effect exceeded the control by 35%. The yeast untreated with ultrasound, but subjected to heat, had the control level values (Bamfort, 2016; Bodrova and Krechetnikova, 2007).

Ultrasound was applied to yeast for 1, 2, 3, 4, 5 and 6 minutes (experiment 1, 2, 3, 4, 5, 6 respectively). The yeast was pitched to the hopped wort until an initial concentration of 20×10^6 per 1 cm^3 was reached. The seed yeast was grown at 14°C. During the growing process, the physiological and biosynthetic activity of the yeast was monitored, namely the total number

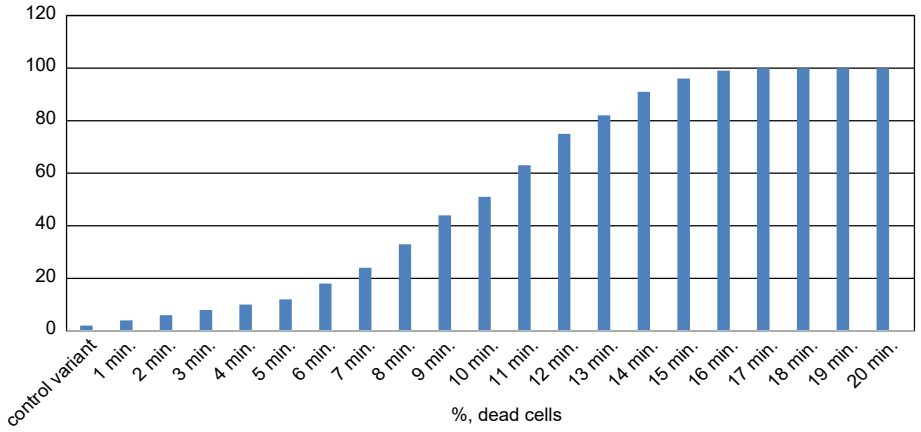

Fig. 1. Number of dead cells, %, related to the duration of ultrasonic treatment

of cells, the percentage of budding cells and glycogen-containing cells.

Yeast provides deep fermentation of wort and produces a distinct aroma and mild flavour in beer. Ultrasound affects the biosynthetic activity of yeast. Figs. 2–4 show the biosynthetic parameters of yeast related to the duration of ultrasonic treatment.

The reproduction rate of yeast is crucial in growing yeast. A 3–4-fold increase in the biomass is considered as a regular rate in brewing. The total number of cells reaches (120–150)×10^6 per 1 cm^3 during the period of active reproduction (Briggs et al., 2004). The initial concentration of cells introduced at the growing seed yeast stage was 20×10^6 per 1 cm^3 in the experimental and control samples. The study revealed that the lag phase lasted at least 24 hours in the control variant and experiments 1, 5 and 6; the rest of the experiments required less than 12 hours. Two-minute ultrasonic treatment ensured the cell concentration of 150×10^6 per 1 cm^3 at 60 hr while the control reached the required concentration at 72 hr. Thus, the yeast growing process shortened by 12 hours.

The number of budding cells is reported in Figure 3. The highest percentage of budding cells (70%) was observed in experiments 1, 2 and 3 at 36 hr, whereas experiments 4, 5, 6 and the control variant

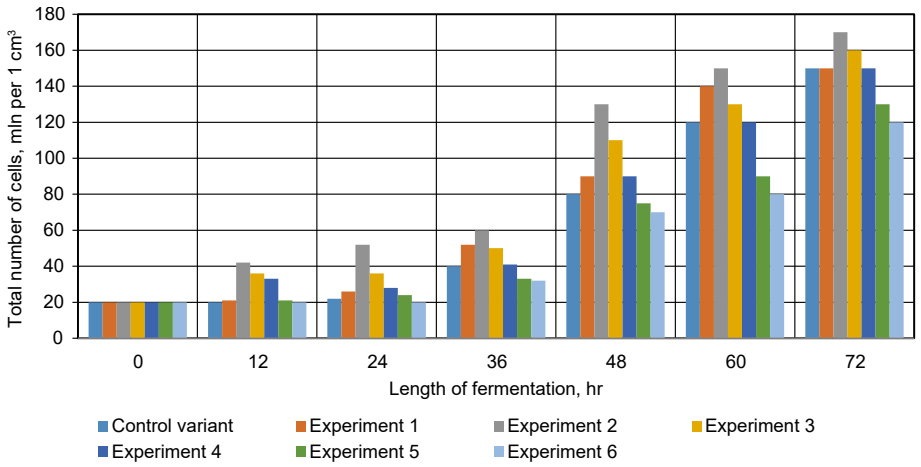

Fig. 2. Total number of cells, mln per 1 cm^3, related to the duration of ultrasonic treatment

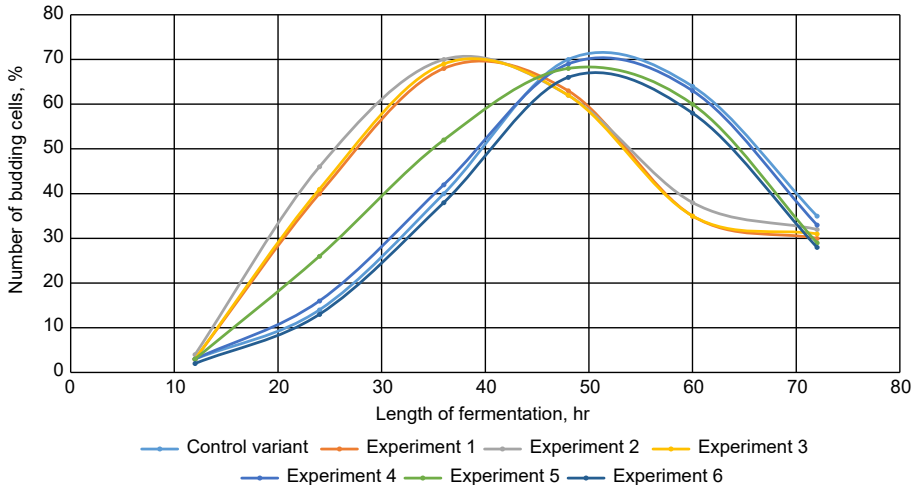

Fig. 3. Percentage of budding cells related to the duration of ultrasonic treatment

reached the value at 48 hr. Thus, experimental variants 1, 2 and 3 showed intensive fermentation and accumulation of budding cells 12 hours earlier than the control variant. This difference indicates a high quality of seed yeast. These findings are confirmed by the number of glycogen-containing cells (Fig. 4). Indeed, experiment 2 showed a 15% increase in the number of glycogen-containing cells (compared to the control variant). The study found that the number of dead cells decreased to 3% compared to 8% in the control variant.

The obtained yeast was then used as seed yeast for the primary fermentation. The physiological state and concentration of yeast play a crucial role in the fermentation of the wort. The initial concentration of cells was 20×10^6 per 1 cm^3 both in the control and experimental samples.

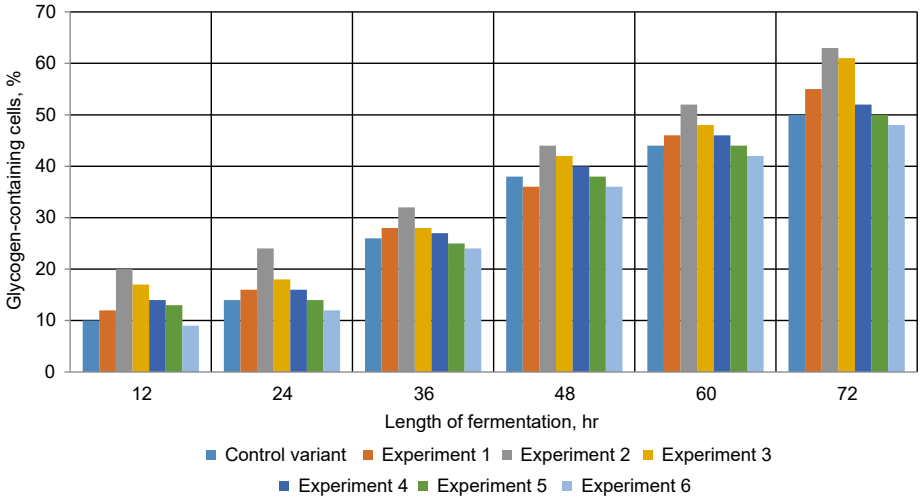

Fig. 4. Number of glycogen-containing cells, %, related to the duration of ultrasonic treatment

Table 1. The amount of carbon dioxide, g, released during fermentation, 100 ml of wort (OG 12%)

Sample	Value
Control variant, no ultrasonic treatment	2.10
Experiment 1, 1 min ultrasonic treatment	2.20
Experiment 2, 2 min ultrasonic treatment	2.70
Experiment 3, 3 min ultrasonic treatment	2.62
Experiment 4, 4 min ultrasonic treatment	2.56
Experiment 5, 5 min ultrasonic treatment	2.26
Experiment 6, 6 min ultrasonic treatment	2.05

The fermentative activity was monitored during the primary fermentation. This technological feature of yeast is essential since it affects the length of primary fermentation and the physical and chemical properties of beer. The fermentative activity was assessed by measuring the carbon dioxide released during the primary fermentation for six days.

Experiment 2 had the highest value of carbon dioxide released compared to the control and other experimental samples. This figure indicates the activating effect of two-minute ultrasound treatment on the yeast cell.

Several researchers from Moscow have examined in detail the effects of ultrasound on the development of *Saccharomyces* yeast. They have found that acoustic vibration frequency can have both positive and adverse effects on yeast development. The study has also proved that the acoustic effect parameters should be optimally adjusted for yeast generation and primary fermentation (Karpenko et al., 2019).

Preparation of yeast extract and its effects on seed yeast

Disruption of cells leads to the release of the cell inner contents into the liquid culture. Yeast hydrolysates and autolysates are valuable since yeast contains biologically active substances (Podpora et al., 2015). Several technologies have been developed to produce high-value protein-amino acid-vitamin products from disrupted yeast cells (Amorim et al., 2016; Jacob et al., 2019a; Tangüler and Erten, 2008; Yang et al., 2021).

A relevant issue is disposal and recycling of food industry waste, of spent brewer's yeast in particular (Ganeva et al., 2020; Marson et al., 2020).

Additional nutrients such as amino acids, ammonium salts, mineral salts and vitamins are added to the wort to increase the rate of reproduction and sugar consumption (Gutierrez, 1993; Jacob et al., 2019c).

This study used ultrasound to produce two types of yeast extract from the spent brewer's yeast *Saccharomyces cerevisiae* by achieving 100% of dead cells in the suspension:

1. YE-1 – extract from yeast suspended in wort
2. YE-2 – extract from yeast suspended in water.

This experiment established the duration of treatment required to obtain extracts by measuring the number of dead cells (Fig. 5).

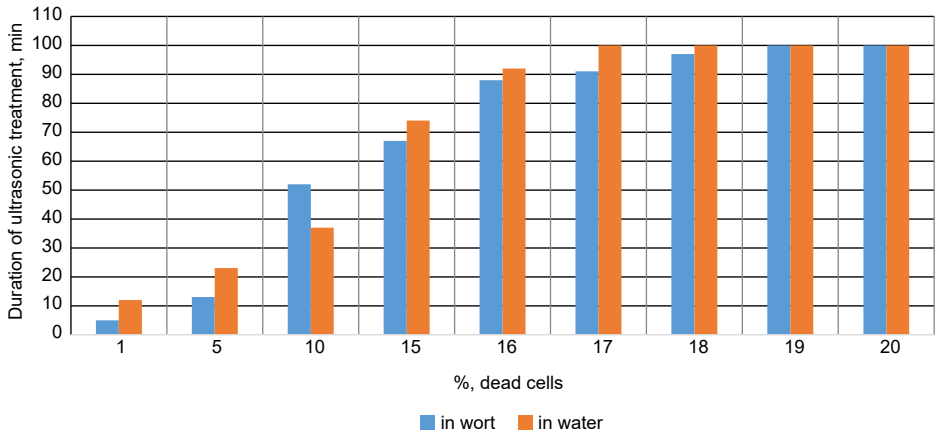

Fig. 5. Time parameters for obtaining yeast extracts

Figure 5 reports that 100% of dead yeast cells dissolved in wort (YE-1) is reached by 19 min of ultrasonic treatment; for the dead yeast cells dissolved in water (YE-2), 100% is achieved by 17 min. Dry substances in wort protect the yeast cell from disruption during ultrasonic treatment. Yeast cells die faster in the water medium.

The obtained yeast extracts were added as 2% of the total volume during seed yeast preparation.

The control variant prepared seed yeast without any yeast extracts.

Experiment 1 prepared seed yeast using YE-1.

Experiment 2 prepared seed yeast using YE-2.

The optimal dose for yeast extract (2% of the total volume) was established in the previous study (Bodrova and Krechetnikova, 2007).

Figs. 6–8 show the relationship between seed yeast biosynthetic parameters and the introduced yeast extracts as growth substances.

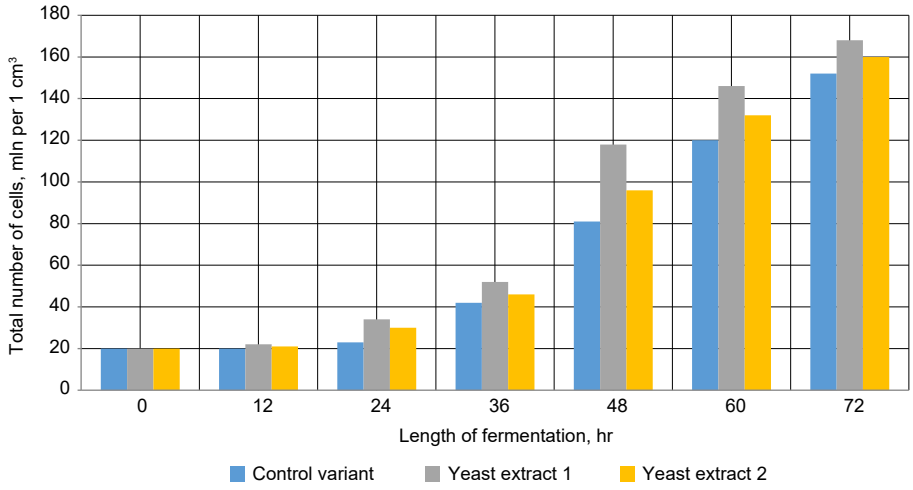

Fig. 6. The total number of cells, mln per 1 cm^3, when yeast extract introduced

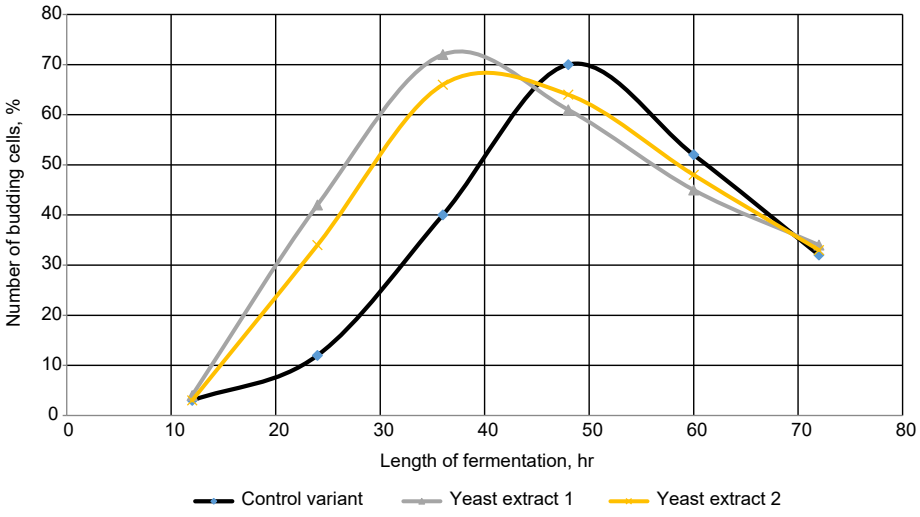

Fig. 7. Stimulation of cell budding by adding yeast extracts

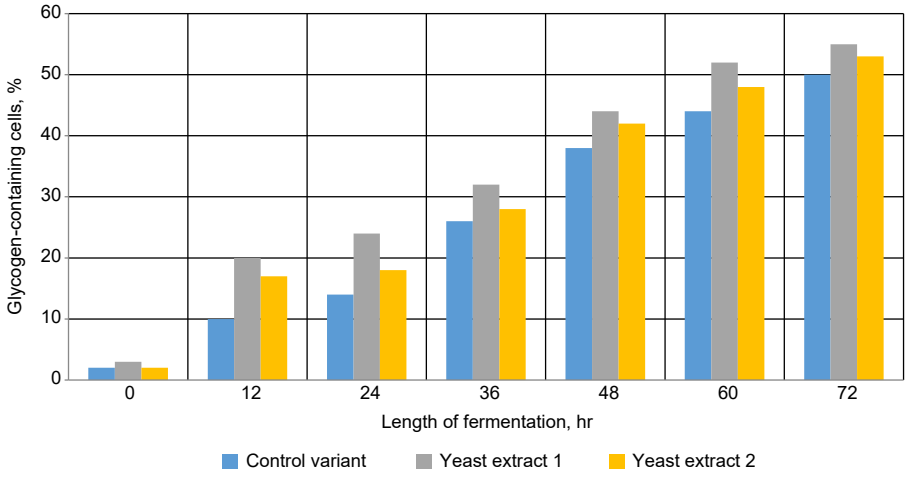

Fig. 8. Glycogen accumulation dynamics when yeast extract introduced

Added yeast extracts (2% of the total volume of wort containing seed yeast) increased the total number of cells. YE-1 and YE-2 ensured the required cell concentrations of 150×10^6 per 1 cm^3 at 66 and 70 hr of yeast growth, respectively. This shortened the process by 6 hours and 2 hours, respectively, compared to the control.

The number of budding cells reached its maximum value after 36 hours of fermentation with YE-1 and YE-2 added. The highest value of budding cells (72%) was observed in the sample with yeast extract YE-1.

All of the experimental variants exhibited accumulation of glycogen in the yeast cells during the seed yeast cultivation. The process dynamics are reported in Figure 8. Glycogen accumulated significantly in the seed yeast containing YE-1. The number of glycogen-containing cells was 55% at 72 hr, while the figure was 50% in the control variant and 52% in experiment 2.

Table 2. B vitamins in yeast extract

Vitamin	Quantitative content, mg/kg
B1 (thiamine)	390
B5 (calcium pantothenate)	160
B6 (pyridoxine)	50
B7 (biotin)	0.08
B8 (inositol)	11 600

Thus, the yeast extract obtained from the yeast suspended in wort greatly intensifies seed yeast preparation.

The activating effect is achieved due to growth substances such as vitamins. The cell cannot independently synthesise some of the vitamins. This study detected B vitamins in the yeast extract obtained from the yeast suspended in wort and the results are presented in Table 2.

The introduction of yeast extract enriches the culture medium with essential vitamins.

The combined effects of ultrasound and yeast extract

The study's final stage examined the combined action of ultrasound and yeast extract YE-1 on seed yeast. To this end, a 20×10^6 per 1 cm^3 concentration of seed yeast was added to the hopped wort. The yeast suspension was treated with ultrasound for two minutes, and yeast extract YE-1 was added (2% of the total volume). The seed yeast was grown for three days. The obtained seed yeast was used in the primary fermentation.

The control sample used seed yeast without ultrasonic treatment or extract.

The experimental sample used seed yeast treated with ultrasound for 2 minutes and contained yeast extract YE-1 (2% of the total volume).

The dry matter decreasing dynamics in the wort during the primary fermentation are demonstrated in Figure 9.

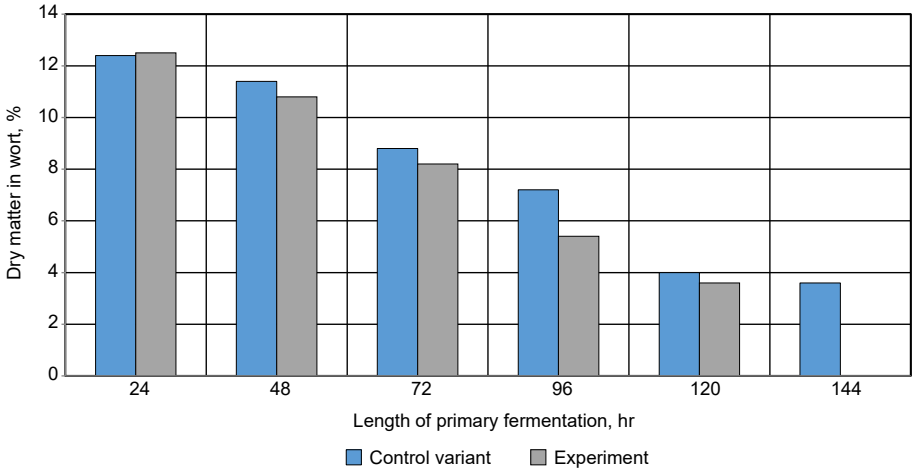

Fig. 9. Dry matter decreasing dynamics in the wort during the primary fermentation

The decrease in dry matter characterises the rate of nutrient uptake by yeast and the fermentation rate. The fermentation rate decreased by 24 hours in the experimental variant with yeast extract.

The physiological parameters of yeast during primary fermentation were analysed. This analysis revealed that on day 6 of fermentation, the percentage of budding cells increased in the experimental variant compared to the control. A higher accumulation of glycogen-containing cells was also found in the experimental sample. The number of dead cells was higher in the control sample than in the experimental at each time interval.

After the primary fermentation was complete the wort was cooled to +0.5°C, allowed to condition for 14 days, and filtered. Then the organoleptic and physico-chemical parameters of beer were evaluated. The profile method was used to assess the taste of the beer. The profile method uses a three-point scale where 0 value indicates the absence of a feature, and 1, 2, 3 values correspond to a slight, distinct and clear expression of the feature (Fig. 10).

The organoleptic profile showed that the beer samples corresponded to the beer obtained from top-fermenting wort (12% original gravity). The samples had a medium flavour and subdued hop bitterness. No sweet, bread, acidic or papery flavours were found in the experimental sample. Instead, it had a slight yeast flavour.

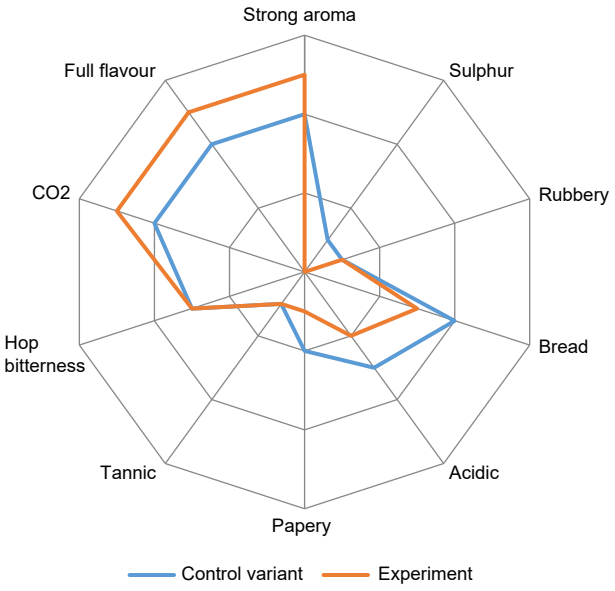

Fig. 10. Organoleptic profile of the samples

The experimental sample won consumer preference. The study found that the beer had a pleasant flavour and aroma compared to the control.

The following physical and chemical parameters were examined in the control and experimental beer samples:

- ethyl alcohol was 4.5 and 4.7 vol.%, respectively
- pH was 4.5 and 4.2

Table 3. Biosynthetic activity of yeast during the primary fermentation

Variant	Length of primary fermentation, hours					
	0	24	48	72	96	120
Number of cells, 10^6 in 1 cm^3						
Control variant	20	120	190	260	310	295
Experiment	20	132	210	310	320	305
Budding cells, %						
Control variant	18	55	60	58	52	42
Experiment	20	65	70	66	60	55
Glycogen-containing cells, %						
Control variant	50	54	68	72	77	82
Experiment	54	66	76	82	85	89
Dead cells, %						
Control variant	22	18	17	14	16	21
Experiment	20	15	13	11	14	16

- head height was 44 and 52 mm
- head retention was 5 and 8 minutes, respectively
- the CO_2 was 0.4 and 0.45%, respectively
- colour was 1.2 and 1.3 units (colour unit is the number of ml of iodine solution 0.1 mol/dm^3 per 100 ml of water).

The beer samples meet the Russian standard requirements for light beer.

The findings are consistent with the data obtained by Choi et al. (2015). They subjected beer samples to ultrasonic treatment during the primary fermentation (Choi et al., 2015).

Original gravity (OG) is a crucial indicator in brewing. It is the amount of dry matter in the wort before pitching the yeast. This indicator affects the flavour of the beer. The higher the OG, the richer the flavour; the lower OG index ensures a lighter and more refreshing flavour. In the study, the OG was 12% in the experimental and control samples. The given original gravity ensured a more pleasant light flavour of the beer with light malt and hop bitterness. Despite the equal original gravity, the experimental samples had a higher fermentation rate due to the activating effect of ultrasound on the yeast cells. This effect increased the alcohol yield by 0.20 vol.% and reduced the length of brewing by 42 hours.

CONCLUSIONS

This study provided a rationale for applying ultrasound to brewer's yeast. It found that ultrasonic treatment for more than 5 minutes increased the number of dead cells to over 10%. For a stimulating effect, the duration of the ultrasonic treatment can be limited to 2 minutes. Further treatment with ultrasound for yeast stimulating purposes is ineffective. The fermentative activity of the treated seed yeast increased compared to the control. The seed yeast preparation process shortened by 12 hours.

The study identified parameters for ultrasonic treatment of spent brewer's yeast, a material used for yeast extract production.

The introduction of yeast extract (2% of the total volume) positively affected the fermentation process. Notably, it shortened the time for preparing seed yeast by 6 hours and improved the physiological state of seed yeast.

This study established the effectiveness of the combined action of two-minute ultrasonic treatment and 2% of yeast extract during seed yeast preparation. This method accelerated the brewing process by 42 hours and improved the quality of the beer.

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