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Alcohol technology intensification with the application of ultrasound

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Abstract

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One of the promising areas of distillery industry successful developments is the acceleration of such processes for the preparation of alcohol as mechanical and enzymatic processing of raw materials, as well as the fermentation of grain wort to improve the quality of the final product. In the article, the effect of ultrasound with an oscillation frequency of 22 kHz and an intensity of 1.0 W/cm^3 (ultrasound) on the water-heat treatment of the wort obtained from the winter triticale grain of the Bashkir short-stalked variety was studied. It is revealed that the ultrasonic effect leads to an increase in the activity of grain α-amylase enzymes. The obtained wort was used for growing seed yeast with simultaneous processing with ultrasound. It is established that this treatment helps to increase the amount of biomass and intensify the process of growing seed yeast. Ultrasonicated wort was fermented with activated yeast, which made it possible to obtain alcohol with a lower content of toxic impurities. As a result of the research a method to intensify the technology of alcohol which was obtained from triticale grain using ultrasound at the stages of water-heat treatment of the wort and growing yeast has been developed. The alcohol yield was 67.3 daL / t of conventional starch.

Keywords: ultrasound; distiller's yeast; triticale grain; grain wort; yeast

Introduction

Distillery industry is one of the branches of food industry, which specializes in the production of alcohol from food raw materials. The main objectives for the development of the alcohol industry are the introduction of resource-saving technologies, improving the alcohol quality, the intensification of various stages of alcohol preparation. To achieve these objectives, such ways of improving the technology as the introduction of progressive methods of water-heat treatment, the improvement of saccharification processes, the intensification of yeast generation and fermentation stages, the introduction of progressive alcohol distillation schemes, the use of enzyme preparations and new types of starchy raw materials are used. The use of triticale grains as a starchy raw material, which is a hybrid of rye and wheat and is the carrier of the best traits of parental forms, is relevant. An important advantage is high yield, nutritional value, resistance to cultivation to adverse climatic conditions and dangerous diseases. High-yielding varieties of triticale Talva-100 and Talva-100-Ido, obtained in the Scientific Research Institute named after Dokuchaev have high starchiness and exhibit significant activity of amylolytic enzymes (Faradzheva et al., 1995; Ismagilov et al., 2018). Denčić et al. (2012) in their research based on an analysis of the economic efficiency of producing bio-ethanol from four varieties of triticale and wheat, four hybrids of corn in the agro-ecological conditions of Serbia, concluded that the costs of producing bio-ethanol from triticale were the lowest; the highest in wheat (Denčić et al., 2012). Studies by Latvian scientists who studied the production of bio-ethanol from various winter wheat, rye and triticale varieties also confirm the fact that triticale grain has a relatively high starch content and low crude protein content, which allows getting a high alcohol yield. The alcohol yield from 1 ton of triticale grain of the "Dinaro" variety was 423.3 liters; the similar indicators for wheat of the "Mulan" variety and rye of the "Matador" variety were 406.8 liters per ton and 370.1 liters per ton (Jansone & Gaile, 2011).

Comparative analysis of the process of fermentation of grain hydrolysates of four wheat varieties and four varieties of triticale with and without the addition of enzyme preparations showed that triticale varieties are characterized by high activity of intact amylolytic enzymes and can be processed into alcohol without the use of enzyme preparations (Pejin et al., 2009).

One of the ways to optimize the processes of grinding, water-heat treatment, yeast generation and fermentation is the effect of ultrasonification of raw materials and yeast.

The world has conducted a large number of studies on the ultrasound effects on food in the processes of extraction, crystallization, freezing, emulsification, filtration and drying (Mason et al., 1996; Gaikwad & Pandit, 2008; Jambrak et al., 2008; Masalimov et al., 2018).

The method of ultrasonic processing is known to precede the hydrolysis and allows us to obtain a greater yield of reducing sugars, reduce the time and temperature of hydrolysis, as well as reduce the acid consumption for hydrolysis in lime saccharification for bio-ethanol technology production (Ashokkumar et al., 2008).

The use of high-intensity ultrasound for food processing is constantly being studied. Studies by Ashokkumar et al. (2008) show that undesirable reactions between the ultrasound-generated radicals and food ingredients can be minimized by choosing lower ultrasonic frequencies for food processing. However, high-frequency ultrasound can also be used to process food, provided that suitable scavengers are present in the solution (John et al., 2019).

Reducing the duration of raw materials preparation for the mechanic and enzymatic method is a hot trend in the alcohol production technology.

One of the ways to solve this problem is the electrical and physical processing of raw materials. Earlier, we carried out research on the effects of ultrasound during the water-heat treatment process of raw materials, namely the study of the effect of ultrasound on enzyme preparations and grain (Smirnova et al., 2007).

We also conducted studies on the effect of ultrasonic processing on yeast suspension and the fermentation process. As a result of these studies, the positive effect of seed yeast, processed with ultrasound, and the intensification of the process of yeast production and fermentation has been established (Bodrova & Krechetnikova, 2007).

The aim of the research described in this article was to develop a method for accelerating such alcohol preparation processes as mechanical and enzymatic processing, as well as fermentation of grain wort obtained from the winter triticale grain of the Bashkir short-stalked variety with the application of ultrasound.

Methods

The studies were conducted in the laboratories of the Department of Technology of Catering and Processing of Vegetable Raw Materials of Bashkir State Agrarian University and Central Analytical Laboratory of the State Scientific Establishment of Bashkir Scientific Research Institute of Agriculture and in the Laboratory of the Fermentation Processes and Industrial Biocatalysis Department of Moscow State University of Food Production.

The objects of the research were:

– triticale grain of Bashkir short-stalked winter variety, class 2;

– *Saccharomyces cerevisiae* alcoholic distiller's yeast of race XII;

Mashes were prepared from low ground grain with a 100% pass through a sieve with a diameter of 1 mm according to the mechanical and enzymatic scheme in laboratory conditions. Hydraulic module adopted 1: 3.5. The aging of the mashes was carried out at t = 450°C for 10 min (peptonizing rest) and at t = 650°C for 35 minutes (self-saccharification rest). During cooking process, we incorporated the enzyme preparation amylosubtilin of submerged cultivation that had the amylolytic activity of 63 units per cm^3 on 1.5 units per gram of grain starch. Glucavamorin of submerged cultivation with glucoamylase activity of 150 units per cm^3 was used for saccharification (Technological Design Standards, 1993; Enzyme Preparations, 2011).

The resulting wort and seed yeast were ultrasonicated in an ultrasonic unit with an oscillation frequency of 22 kHz and an oscillation intensity of 1.0 W / cm^3.

Methods of research:

– the amino acid composition of the wort was determined on the amino-analyzer "BIOTRONIK";

– the total number of cells was calculated on a special counter "SPARE PART LIST" Model 902 Yeast-2004;

– alcohol impurities were determined on an HP 6850 Agilent Series Gc Sustem gas chromatograph (Gas and Chromatographic Express-Method, 2013);

– the quality of triticale grain was determined according to the requirements of GOST (Triticale, 2017);

– the physicochemical parameters of wort and fermented alcohol were carried out by standard methods in the technology of alcohol (Technoclinical and Microbiological Control of Alcohol Production Instruction, 2007);

– vitamins were determined according to the approved GOST (Premixes, 2012).

Statistical analysis of the results was carried out using computer software from StatSoft (Statistica 10.0). The tables and figures show the mean values of 3-x biological replicate.

Results and Discussions

In our studies, we used a new high-yielding hybrid of winter triticale grain of the Bashkir short-stalked variety class 2, selected by the State Scientific Establishment of Bashkir Scientific Research Institute of Agriculture with a starch mass fraction of 64.5%. Physical and chemical indicators of grain triticale taken for research are presented in Table 1.

Table 1. Physical and chemical indicators of triticale grain

Parameter name	GOST requirements 34023-2016	Results
Colour	Typical to triticale grain	Applicable
Scent	Typical to triticale grain	Applicable
Grain unit, gram per liter, min	680	700
Moisture, % max	14	14
Gluten, % min	18	19
Black dockage, K max	2	2
including mineral (impurity)	0.3	0.2
Pebble, slag, ore	0.1	0.1
Cockle	0.5	0.3
Spoiled grain	0.5	0.5
Starch content, %	Not applicable	64.5

Winter triticale grain of the Bashkir short-stalked variety meets the requirements of the Russian standard GOST 34023-2016. It should be noted that in the alcohol industry, the quality of grain, which is used for cooking, is not regulated. It is allowed to apply grain of any degree of defectiveness.

At the initial stage of the research, grain wort processed with the ultrasound with a 22 kHz oscillation frequency and 1.0 W/cm^3 intensity. Grain wort that was not treated with ultrasound was used as control wort. The results of the study are presented in Table 2.

Table 2. Changes in wart indicators due to ultrasonic exposure

Ultrasound processing time, minutes	Mass fraction of dry substances,%	Mass concentration of fermentable carbohydrates,%	Titratable acidity cm^3 NaOH conc. 0.1 mol / dm^3 per 20 cm^3 of wart	mActive acidity pH
control	16.8	14.8	2.80	5.50
10	16.8	14.8	2.81	5.50
20	16.8	15.1	2.82	5.60
30	16.9	15.2	2.84	5.60
40	16.9	15.4	2.95	5.70
50	17.0	15.4	2.95	5.70
60	16.9	15.3	3.04	5.80
70	16.5	14.0	3.04	5.80
80	16.2	13.6	3.10	6.00
90	16.0	13.0	3.20	6.10

From Table 2 it can be seen that during the process of ultrasonic treatment of the wort, the titratable and active acidity of the medium from the 10th to the 80th minute of the treatment changes from 2.8 to 3.2 (by the amount of NaOH concentration, 0.1 mol / dm^3 per 20 cm^3 of wort) and with a pH from 5.55 to 6.10, respectively. The mass fraction of dry substances and fermentable carbohydrates from 10th to 50th minutes of exposure increases from 16.8 to 17.0% and from 14.8 to 15.4%, respectively. Further processing causes a sharp decrease in the amount of dry substances and fermentable carbohydrates, which presumably occurs as a result of inactivation of hydrolytic enzymes. Kadkhodaee & Povey (2008) also studied the effect of the inactivation of α-amylase enzymes produced by the *Bacillus amyloliquefacience* bacterium, under the exposure of an ultrasonic frequency of 30 kHz at t = 50°C (Kadkhodaee & Povey, 2008). Then the duration of ultrasonic exposure activity of grain was studied. The results are presented in Figure 1. It has been established that with an increase in the duration of ultrasonic exposure, the activity of α-amylase reached its maximum value at the 50th minute of treatment, α-amylase at the 40th minute, and proteolytic activity increased at the 20th minute. A further increase led to a decrease in enzyme activity.

Similar results are obtained by Pejin et al. (2012) in whose studies it was found that as a result of precure of the wort with ultrasound for 5 minutes at t = 60°C, the saccharification and fermentation process increases the yield of glucose and maltose by 15.71% and 52.57%, respectively, and allows increasing the content bio-ethanol by 10.89% and reduce the process from 72 to 48 hours (Pejin et al., 2012). Then, we conducted research on the effect of the duration of

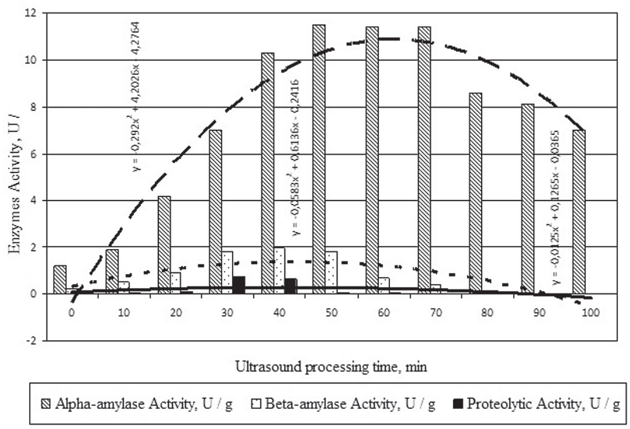


Fig. 1. The dependence of the duration of ultrasound on the activity of α-amylase, β-amylase and proteolytic activity of the grain

ultrasound on the quality of the wort with the enzyme preparation added in an amount of 1.5 U/g of conventional starch at a temperature of 67-74°C. The control version was prepared in a similar way, but not exposed to ultrasonic waves. Studies have shown that the maximum content of reducing sugars was detected with a total duration of ultrasonic exposure for 50 min (Figure 2), which is higher than the control by 22%. The total duration of the wort preparation was 150 min, which is 35 min less than in the control. Further processing according to the obtained research was ineffective.

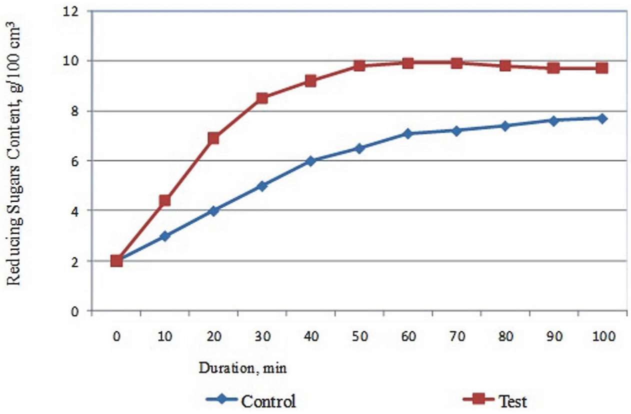


Fig. 2. Change of reducing sugars in the process of ultrasonic treatment of boiled mass

Analysis of the boiled mass for the accumulation of starch hydrolysis products showed a slight change in maltodextrins, which is 1.0% higher than the dry matter of the wort than in the control. And the increase in mono-disaccharides by 9.2% of the wort solids is higher than in the control (Table 3).

Table 3. The effect of ultrasonic processing of boiled mass on the accumulation of starch hydrolysis products

The name of the products of starch hydrolysis	Content, % of dry matter wart	
	Control	Test
Mono-disaccharides	62.3	71.5
Maltodextrins	8.2	9.2
Achrodextrins	2.4	2.1
Amylodextrins	0.8	0.6
Erythrodextrins	1.0	1.0

The next stage of research was the development of a method for accelerating the water-heat treatment of wort made from triticale grain with continuous ultrasonic exposure on the cooked mash. For this, the processing of cooked mash with ultrasound was carried out continuously using α-amylase at a temperature of 95°C for 40 minutes. The saccharification was carried out at low temperature.

Studies have shown that the maximum content of reducing sugars was observed when processing ultrasound for 40 min (Figure 3). The sugar content in the experimental variant is 77% higher than in the control. Further ultrasonical exposure did not give a change in the content of reducing sugars.

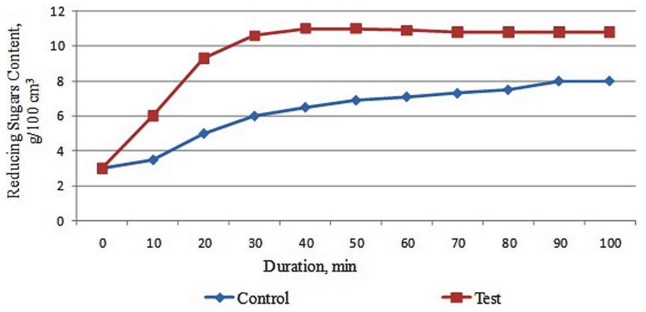


Fig. 3. Change of reducing sugars in the process of continuous ultrasonic processing of boiled mass

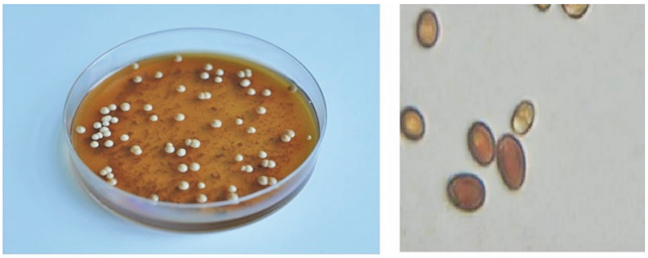
Also, our studies have shown that ultrasonic exposure allows us to save the content of vitamins in the wort (Table 4).

Studies have shown that the continuous mode of ultrasonic exposure improves the quality of the wort, accelerates

Table 4. The effect of ultrasonic exposure of mass in continuous mode on the content of vitamins

Vitamin	Content, mg/100 cm³	
	Control	Test
Nicotinic acid	5.2	5.2
Biotin	0.11	0.10
Thiamin	0.40	0.39
Pantothenic acid	0.10	0.10
Riboflavin	0.28	0.28

the process of obtaining alcohol for 120 min and allows us to reduce the dose of the enzyme preparation by 45%. After the preparation and processing of the wort with ultrasound we began the process of fermentation, which is directly related to the physiological and morphological properties of the yeast used. The higher is their ability to ferment, the faster is the fermentation process takes place and the greater is the amount of alcohol yield. In our previous studies, we found that ultrasonic processing of the yeast for 4 min has a positive effect on the physiological activity of the yeast (Bodrova & Krechetnikova, 2007). In connection with this fact, in further studies, the yeast suspension was treated for 4 minutes with an oscillation frequency of 22 kHz and an intensity of 1.0 W/cm^3. Microscope photos of yeast under an ultrasonic exposure and pure culture yeast are presented in Figure 4.



a) *b*)

Fig. 4. *a*) Pure culture of *Saccharomyces cerevisiae*; *b*) *Saccharomyces cerevisiae* yeast under a microscope with a magnification of 1000 times

Seed yeast treated with ultrasound was set in the wort in the amount of 10% of the total volume. Fermentation was carried out in a thermostat at a temperature of 28-32°C. The process was controlled by the total number of cells, by fermentation activity and the amount of unfermented carbohydrates. Also at the end of the fermentation process we studied the indicators of fermented wash.). The test results are shown in Figures 5-8 and in Table 5.

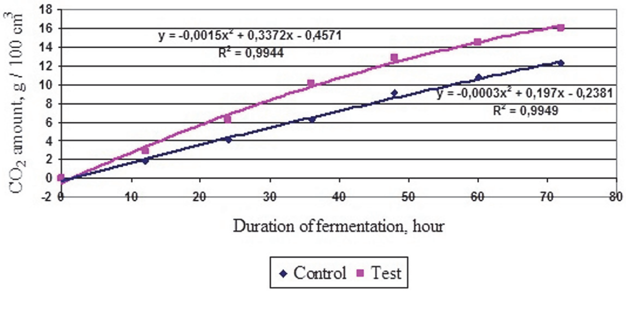


Fig. 5. Dynamics of fermentation activity during wort fermentation with ultrasound activated yeast

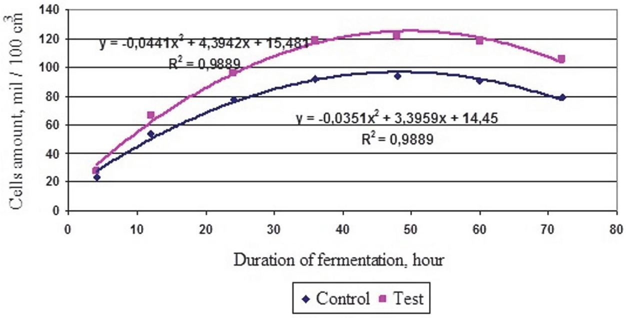


Fig. 6. The content of the total number of cells during the wort fermentation with ultrasound activated yeast

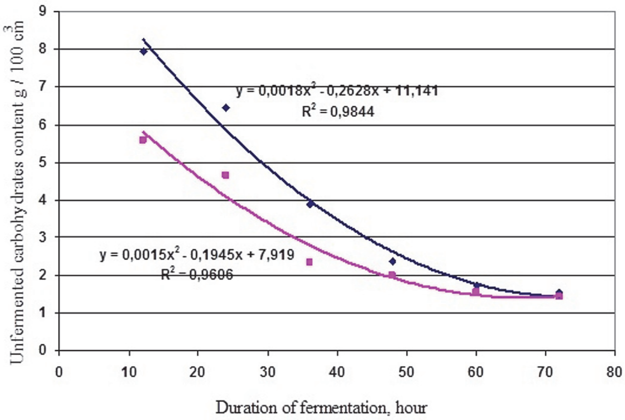


Fig. 7. Content of unfermented carbohydrates during fermentation of the wort with ultrasound activated yeast

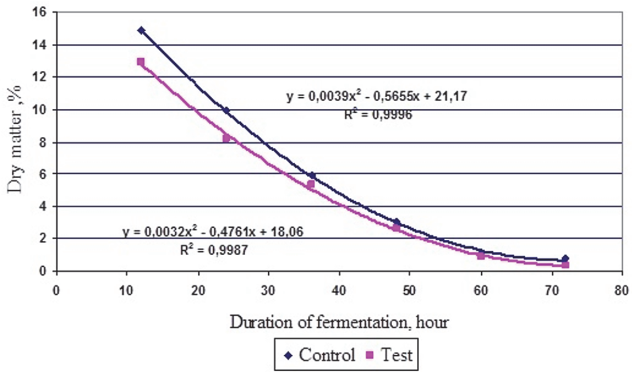


Fig. 8. The dry matter content during wort fermentation with ultrasound activated yeast

From Figures 5-8, it can be seen that the fermentation activity in the test sample exceeds control by 29%, the total number of cells by 33%, and the content of non-fermented by 0.15 g / 100 cm^3. The duration of fermentation is reduced by 6-8 hours.

Table 5. Physical and chemical indicators of fermented wash

Indicators	Control	Test
Mass fraction of carbohydrates (%): total soluble	0.62 0.49	0.43 0.40
Mass fraction of undissolved starch,%	0.16	0.12
Active acidity, pH	4.82	4.76
Volume fraction of alcohol,%	8.01	8.92

From Table 5 it can be seen that in the test sample, the indicators of fermented wash are improving, namely: the mass fraction of total carbohydrates decreases by 0.19% compared with the control and the volume fraction of alcohol increases by 0.91%.

Then the fermented wash of the experimental and control sample was subjected to distillation to determine the content of alcohol and by-products by gas chromatographic analysis (Table 6).

Table 6. Gas chromatographic analysis of the wash distillate

Impurities	Content, mg/dm^3	
	Control	Test
Propanol-1	115.5	104.9
Propanol-2	0	0
Butanol -1	8.7	4.6
Butanol -2	0	0
Isobutanol	877.9	574.1
Isoamylol	2297.6	1739.8
Acetaldehyde	486.0	279.2
Methyl acetate	75.2	46.9
Ethyl acetate	156.9	96.,2
Total impurities	4017.8	2845.7
Methanol, vol. %	1.95	1.42

From Table 6 it can be seen that the total content of impurities in the test sample decreased by 1172.1 mg/dm^3 compared with the control one. During wort fermentation with yeast seed activated by ultrasound, the duration of fermentation is reduced by 8 hours, the physical and chemical characteristics of the fermented wash are improved, the alcohol content is increased and its quality is also improved.

Conclusion

In the studies, a new high-yielding hybrid of winter triticale grain of the Bashkir short-stalked variety class 2, selected by State Scientific Establishment Bashkir Scientific Research Institute of Agriculture was used. It is established that this hybrid meets the GOST requirements of 34023-2016, namely, it has a mass fraction of starch 64.5%, grain unit 700 g/l and humidity 14%. We subjected obtained wort with ultrasonic processing with an oscillation frequency of 22 kHz and an intensity of 1.0 W/cm^3 and found that during the ultrasonic processing of the wort, the titrated and active acidity of the medium from the 10t^{h} to the 80th minute of processing changes from 2.8 to 3.2 and with a pH of 5.55 to 6.10, respectively. The mass fraction of dry matter and fermentable carbohydrates from the 10th to the 50th minutes of exposure increases from 16.8 to 17.0% and from 14.8 to 15.4%, respectively. Further processing causes a sharp decrease in the amount of dry matter and fermentable carbohydrates, which presumably occurs as a result of inactivation of hydrolytic enzymes. The duration of ultrasonic effect on the grain enzymes activity was studied. It was established that with an increase in the duration of ultrasonic processing, the activity of α-amylase, b-amylase and proteolytic activity increased and reached a maximum value at the 50th, 40th and 20th minutes, respectively. A further increase led to a decrease in enzyme activity.

We studied the duration of ultrasonic effect on boiled mass in a discrete mode on the wort quality and found out that the highest content of reducing sugars was detected at the 50th minute of processing, which is higher than in the control by 21-22%. The total duration of wort preparation was 160 min, which is 40 min less than in the control. Further processing did not give any additional effect. It was established that ultrasonic processing of the mass made it possible to preserve the content of vitamins in the wort. We also studied the effect of ultrasonic processing on seed yeast and established that this processing contributes to an increase in biomass synthesis and intensification of the growing seed yeast process. At the same time, the fermentation activity in the test sample exceeds control by 29%, the total number of cells by 33%, and the content of unfermented sugars decreases by 0.15 g /100 cm^3. The duration of fermentation is reduced by 8 hours. Also in the test sample, the indicators of fermented mash are improved, namely, the mass fraction of total carbohydrates is reduced by 0.19% compared to the control and the volume fraction of alcohol increases by 0.91%. It was revealed that the total content of impurities in the test sample is reduced by 1172.1 mg/dm^3 compared with the control. The alcohol yield is 67.3 daL/t of conventional starch.

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