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SELECTION OF THE COMPLEX OF ENZYME PREPARATIONS FOR THE HYDROLYSIS OF GRAIN CONSTITUENTS DURING THE FERMENTATION OF THE WORT OF HIGH CONCENTRATION

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Abstract. In this paper, an optimal complex is selected of enzyme preparations for hydrolysis of the components of grain raw materials during fermentation of high concentration wort. When selecting enzyme systems, their effect on the technical and chemical parameters of the fermented wash during the fermentation of wort is investigated. For the research, maize grain with a starch content of 69.0% was used. Fermentation was carried out with 18–30% of dry matters (DM) in the wort, using the osmophilic yeast strain Saccharomyces cerevisiae DO-16.The recommended concentration of the enzyme preparation Amylex 4 T (the source of the α-amylase enzyme) – 0.4–0.6 units of α-amylase ability/g of starch – is optimal for the concentration 18–27% of DS in the wort. For 30% of DS, it is practical to use 0.6 units of α-amylase ability/g of starch. With the use of the enzyme preparation Diazyme TGA (the source of the enzyme glucoamylase), the value is 7.5 units of glucoamylase ability/g of starch, alcohol accumulation in fermented washes was 10.51, 13.35, 15.78% vol., according to the wort concentrations 18, 27, 30%, respectively. It has been established that with the application of the cytolytic enzyme Laminex 750, the concentrations of dissolved carbohydrates and non-dissolved starch have a tendency to decrease.

In the samples where the proteolytic enzyme preparation Alphalase AFP was added at a concentration of 0.05 units of proteolytic ability/g of raw materials, there was an increase in the accumulation of yeast cells by 6.5% compared with the reference sample. The recommended concentration of Deltazyme VR XL (the source of β-glucanase and xylanase) is 0.05 units β-glucose/g of raw materials. The addition of a cytolytic and proteolytic enzyme preparation in combination with β-glucanase and xylanase contributed to an increase in the accumulation of ethanol in the washes by 1.7% compared with the reference sample, and to an almost 33% decrease in the concentration of dissolved carbohydrates and non-dissolved starch. On the basis of experimental studies, it has been found that using a complex of enzyme preparations – amylolytic (Amylex 4T), saccharifying (Diazyme TGA), proteolytic (Alphalase AFP), cytolytic (Laminex 750), and complex AF β-glucanase and xylanase (Deltazyme VR XL), in various combinations of their concentrations, – contributed to the intensification of the fermentation process of the wort and increased accumulation of the target product, ethanol, by 0.8–1.4%, depending on the wort concentration. The highest amount of ethanol accumulated at the maximum dosage of additional enzyme preparations.

Key words: enzyme preparation, high concentrated wort, starch, dry matter, fermentation.

ПІДБІР КОМПЛЕКСУ ФЕРМЕНТНИХ ПРЕПАРАТІВ ДЛЯ ГІДРОЛІЗУ СКЛАДОВИХ ЗЕРНА ПРИ ЗБРОДЖУВАННІ ВИСОКОКОНЦЕНТРОВАНОГО СУСЛА

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Анотація. У роботі підібрано оптимальний комплекс ферментних препаратів для гідролізу складових зернової сировини при зброджуванні сусла високих концентрацій. При підборі ферментних систем досліджено їхній вплив на техніко-хімічні показники зрілих бражок при зброджуванні сусла. Для досліджень використовували зерно кукурудзи крохмалистістю 69,0%. Зброджування сусла проводили при концентрації сухих речовин сусла 18–30%, з використанням осмофільного штаму дріжджів Saccharomyces cerevisiae DO-16. Концентрація ферментного препарату Амілекс 4Т (джерело ферменту α-амілази) 0,4–0,6 од. АЗ/г крохмалю є оптимальною для замісу сусла концентрацією 18–27% СР, а для 30% СР доцільно використовувати 0,6 од АЗ/г крохмалю. При застосуванні ферментного препарату Діазим TGA (джерело ферменту глюкоамілази) – 7,5 од. ГлЗ/г крохмалю, накопичення спирту в зрілих бражках було 10,51; 13,35; 15,78% об. відповідно до концентрацій сусла 18, 27, 30%. Встановлено, що при застосуванні цитолітичного ферментного препарату Ламінекс 750 спостерігалася тенденція зниження концентрацій розчинених вуглеводів та нерозчиненого крохмалю.

У зразках, де вносили протеолітичний ферментний препарат Альфалаза AFP концентрацією 0,05 од. ПрЗ/г сировини, спостерігалося збільшення накопичення дріжджових клітин на 6 , 5 % порівняно з контролем. Рекомендована концентрація Дельтазим VR XL (джерело β-глюканази і ксиланази) – 0,05 од β ГлЗ/ г сировини. Додавання цитолітичного та протеолітичного ферментного препарату в комплексі з β-глюканазою і ксиланазою сприяло підвищенню накопичення етанолу в бражках на 1,7% порівняно з контролем та зниженню концентрації розчинених вуглеводів і нерозчинного крохмалю майже на 33%.

Ключові слова: ферментний препарат, висококонцентроване сусло, крохмаль, сухі речовини, зброджування.

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Introduction. Formulation of the problem

The technology of ethyl alcohol obtained from starch-containing raw materials is based on the enzymatic catalysis of high-molecular-weight polysaccharides of the processed substrate to produce carbohydrates that are yeast-fermented into ethyl alcohol.

The presence of components of incomplete hydrolysis of raw materials creates the precondition of local stagnant zones, especially in high concentrated wort, where contaminating microflora develops.To maximise hydrolysis of all components of the raw material by means of enzyme preparations, it is necessary to select correctly the quantitative and qualitative composition of the processed grain, as well as to determine the concentration of enzyme preparations.

Analysis of recent research and publications

One of the ways to intensify the fermentation of the wort is to increase the concentration of dry matter in it. It will increase the productivity of the fermentation room, and reduce the cost of energy resources [1-3]. However, the application of the fermentation technology of high concentration wort gives rise to a number of problems associated with increased viscosity of the mash, and limited hydrolysis of the raw material components [4-6]. That is why, in order to increase the efficiency of biotechnological processing starchy raw material, a necessary condition is the selection of an optimal complex of enzyme preparations for the hydrolysis of grain constituents when fermenting wort of high concentrations [7-10].

The role of amylolytic enzyme preparations is in their catalytic functions of hydrolysis of starch (rarefaction, dextrinisation, saccharification) [11]. Along with amylolytic ones, other enzyme preparations are required that provide hydrolysis of non-starch polysaccharides (cellulose, hemicellulose) that create around the starch an impermeable envelope for α-amylase and glucoamylase [12-13]. Application of such enzyme preparations makes for deeper hydrolysis of starch from raw materials by amylolytic enzymes. Besides, it results in an additional amount of fermented sugars due to hydrolysis of cellulose [14-16].

Another important component of raw materials is protein. When processing starchy raw materials by the high-temperature scheme, most of the protein is destroyed, and with the low-temperature one, the role of the protein increases. That is why, raw protein can be a potential source of amino acids. The negative effect of the presence of protein is its accretion on the equipment, which makes the wort likelier to be infected. The formation of protein complexes with starch makes the latter unavailable for amylase, which reveals in the raw material losing more carbohydrates. So, hydrolysis of proteins when using proteases at low-temperature schemes to process grain raw materials is an important problem [17-18].

It is a practical and topical task to select enzyme complexes for the hydrolysis of grain constituents when fermenting highly concentrated wort, since this will intensify the fermentation of the wort, reduce the viscosity of the mash, and increase the output of alcohol.

The **aim** of the work was to research and select the complex of enzyme preparations necessary for the hydrolysis of grain constituents during thermo-enzymatic treatment and fermentation of the wort of high concentrations.

Objectives of the research:

1. Determine the concentration of amylolytic enzyme preparations in the fermentation of the wort of high concentrations;
2. Determine the concentration of auxiliary enzyme preparations (proteases, cellulases, xylanase).

Research Materials and Methods

For the study, milled maize grains were used, with the 100% dispersity of passing through a sieve with the mesh diameter 1 mm. The wort was fermented by the osmophilic, thermotolerant strain of Saccharomyces cerevisiae DO-16.

Enzyme preparations of the Danisco company were used. Amylex 4T (with the activity 1158 units/cm³) and glucoamylase Diazyme TGA (with the activity 11152 units/cm³) were used as α-amylase. The enzyme preparations were added by units of activity. The starch content of the maize grain used was 69.0%. The thermo-enzymatic treatment of the starch-containing raw material was carried out at a temperature of 90–92 °C for 3 hours, and the saccharification of rarefied dough at 50–55 °C for 30 min. The concentration of thermostable α-amylase was 0.4; 0.60 units of α-amylase ability/g of starch. That of glucoamylase was 5.0; 7.5; 10 units of glucoamylase ability/g of starch.

In the experiments, the cytolytic enzyme preparation Laminex 750, the proteolytic Alphalase AFP (Danisco) and the Deltazyme VR XL (WissBioTech) (as a source of β-glucanase and xylanase) were used. In the course of studies, the concentration of the proteolytic enzyme preparation Alphalase AFP was 0.02; 0.028; 0.035 units of proteolytic ability/g of raw materials, cytolytic Laminex 750–0.125; 0.25; 0.35 units of cytolytic ability/g of raw materials, and PP Deltazyme VR XL 0.05; 0.07; 0.1 units of β-glucose ability/kg of raw materials.

The mash was fermented at 32–35 °C, with the concentrations of wort dry matter 18, 25, 27, and 30%, using the osmophilic yeast strain Saccharomyces cerevisiae DO-16. The yeast inoculum was added in the proportion 20–30 mln/cm³ of the wort, depending on

the concentration of the mash.

The starch content in the initial grain was estimated by Evers's method [20], the grain humidity by drying to constant weight [19]. The granulometric composition of the milled grain was determined by sizing on metal and nylon 6 sieves [19]. The dry matter concentration was determined with a saccharimeter and a refractometer [20].

The total number of yeast cells in 1 cm³ was determined by direct count in Gorjaev's chamber. In the laboratory, the wort was fermented by the fermentation test method in conical flasks with sulphuric acid valves in a thermostat at a temperature of 30–35°C. The dynamics of carbon dioxide emission was controlled by weight measurement [19]. In the fermented wash, the pH was determined with the device PH-150 MI, the ethanol content by the picnometer method, the soluble and alcohol-soluble carbohydrates, insoluble starch, and dextrins by the photoelectrocolorimetric method with the anthrone reagent [19].

Results of the research and their discussion

Since the effectiveness and intensity of fermentation, as well as the amount of alcohol, are largely dependent on the carbohydrates content in the wort, our research was aimed at determining the optimal concentration of amylolytic enzyme preparations, which would provide a high degree of hydrolysis of the starch in the raw material to fermentable sugars while fermenting high-concentrated wort.

The research has been carried out to determine the effect of the dry substances concentration in the wort on the amount of the enzyme preparation α-amylase spent and on the duration of rarefying the mash.

As can be seen from Fig. 1 (a–d), with an increase in the α-amylase doses from 0.075 to 0.8 units of α-amylase ability/g of starch, regardless of the wort concentration, the content of dissolved carbohydrates gradually increases. An analysis of the data has shown that the α-amylase doses 0.4–0.6 units of α-amylase ability/g of starch is optimal for a mash with the dry matter concentration 18–27%, and for 30%, 0.6 units of glucoamylase ability/g of starch should be used. But, as you can see from Fig. 1 (a–d), the degree of hydrolysis of starch of the raw materials, no matter how concentrated the wort is, depends on the duration of rarefaction. So with a 1.5-hour dilution, the content of dissolved carbohydrates is 11.4; 11.78; 12.8; 13.0 g/100 cm³ depending on the concentration of the wort. With an increase in the duration of the rarefication to 3 hours, this parameter increases by 9.0; 9.42; 16.5; 22.3%, respectively, and with the further prolongation to 4 hours, it practically does not change. Thus, the duration of the thermo-enzymatic treatment was 3 hours. At the 18% concentration of dry substance in the maize mash, the duration of the rarefaction can be reduced to 2–2.5 hours.

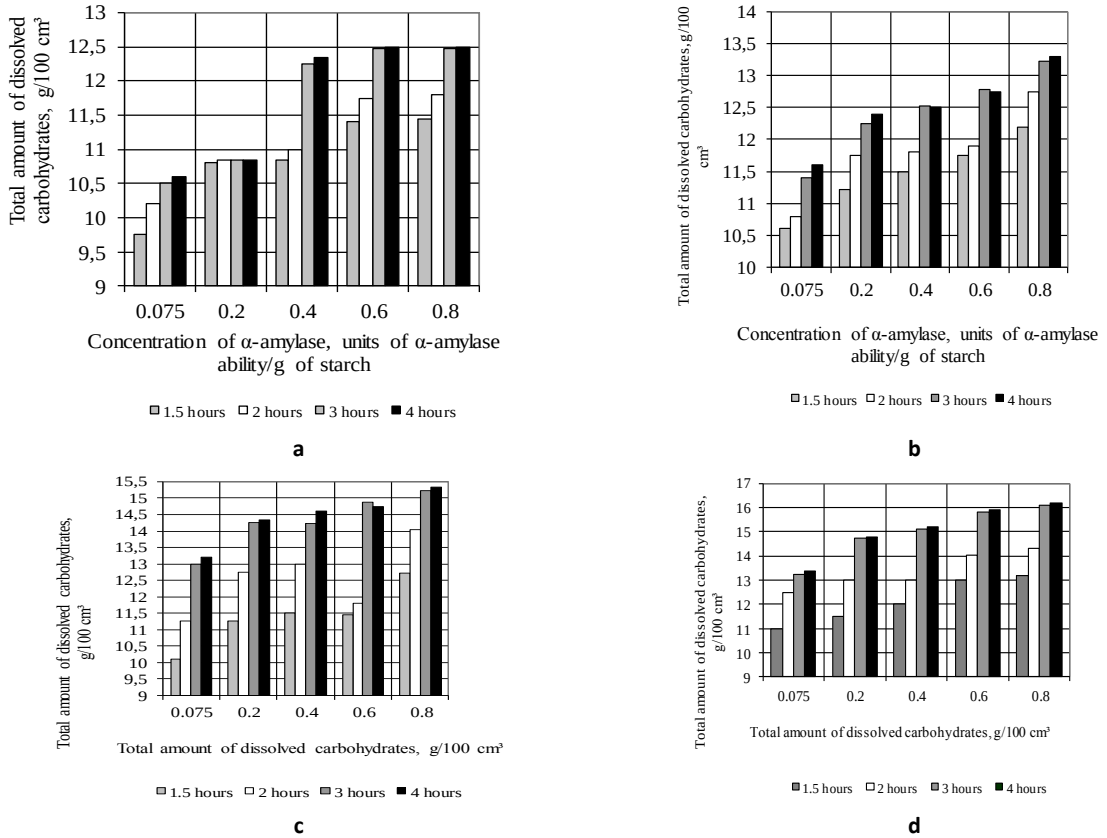

Fig. 1. The effect of the concentration of amylolytic enzyme preparations on the parameters of maize hydrolysis: a – DS concentration 18%, b – DS concentration 25%, c – DS concentration 27%, d – DS concentration 30%.

To confirm the data obtained, studies have been carried out on the fermentation of maize wort with DS concentrations 18, 25, 27, 30% (Table 1).

In the experiments, for saccharification of the rarefied mash, glucoamylase was used in the proportion 7.5 units of glucoamylase ability/g of starch. The saccharification was carried out at a temperature of 50–55°C, for 0.5 hours.

The values obtained during the wort fermentation have confirmed the enzyme preparation doses suggested earlier. This can be seen from the data both on the content of dissolved carbohydrates and on the alcohol synthesis in washes, which are at the prescribed level.

Table 1 – Chemical and technological parameters of fermented wash depending on the quantitative composition of amylolytic enzyme preparations and wort concentrations

№	[DS],%	[α-amylase], units of α-amylase ability/g of starch	∑ CO_2, g/200cm³	Acidity, degrees	Content of non-fermented carbohydrates, g/100 cm³		Alcohol content,% vol.
					Dissolved	Non-dissolved starch	
1	18	0.075	16.20±0.1	0.44±0.1	0.480±0.01	0.120±0.01	9.80±0.01
2	18	0.200	16.90±0.1	0.44±0.1	0.360±0.01	0.120±0.01	10.55±0.01
3	18	0.400	17.00±0.1	0.44±0.1	0.250±0.01	0.100±0.01	10.66±0.01
4	18	0.600	17.60±0.1	0.45±0.1	0.230±0.01	0.085±0.01	10.68±0.01
5	18	0.800	17.80±0.1	0.44±0.1	0.210±0.01	0.081±0.01	10.70±0.01
6	25	0.075	17.92±0.1	0.43±0.1	0.490±0.01	0.110±0.01	12.18±0.01
7	25	0.200	18.40±0.1	0.44±0.1	0.450±0.01	0.100±0.01	12.28±0.01
8	25	0.400	19.00±0.1	0.44±0.1	0.380±0.01	0.090±0.01	12.40±0.01
9	25	0.600	19.17±0.1	0.43±0.1	0.260±0.01	0.085±0.01	12.48±0.01
10	25	0.800	19.22±0.1	0.44±0.1	0.240±0.01	0.080±0.01	12.52±0.01
11	27	0.075	19.30±0.1	0.44±0.1	0.580±0.01	0.160±0.01	13.20±0.01
12	27	0.200	19.40±0.1	0.44±0.1	0.500±0.01	0.120±0.01	13.22±0.01
13	27	0.400	19.60±0.1	0.43±0.1	0.360±0.01	0.099±0.01	13.41±0.01
14	27	0.600	19.90±0.1	0.43±0.1	0.320±0.01	0.095±0.01	13.45±0.01
15	27	0.800	20.00±0.1	0.43±0.1	0.310±0.01	0.095±0.01	13.45±0.01
16	30	0.075	19.00±0.1	0.44±0.1	0.850±0.01	0.260±0.01	15.20±0.01
17	30	0.200	20.65±0.1	0.43±0.1	0.750±0.01	0.250±0.01	15.26±0.01
18	30	0.400	21.30±0.1	0.43±0.1	0.600±0.01	0.220±0.01	15.34±0.01
19	30	0.600	21.50±0.1	0.43±0.1	0.580±0.01	0.160±0.01	15.40±0.01
20	30	0.800	21.70±0.1	0.43±0.1	0.570±0.01	0.150±0.01	15.45±0.01

For the quality of the bioconversion of the wort, a necessary condition is the degree of hydrolysis of the constituents of the rarefied dough to the digestible carbohydrates. Therefore, studies have been carried out on the effect of the concentration of enzyme preparation glucoamylase on the bioconversion of the wort of high concentrations (Table 2). At concentrations of wort, 18% DS received a mashkin with excellent indices of dissolved carbohydrates (0.23–0.25 g/100 cm³ march) and insoluble starch (0.08–0.07 g/100 cm³ march). With an increase in the concentration of wort, these rates increased and at concentration of 27% DS the content of dissolved carbohydrates increased almost 2 times, and the alcohol content in the marsh was 13.29–13.36%. At concentrations of dry substances, the content of dissolved carbohydrates in barges increased by 30% to 0.61–0.65 g/100 cm³ march , and insoluble starch exceeded the regulated values by 10–20%. Accumulation of alcohol in mature jugs amounted to 15.75–15.78% vol. (Table 2).

Table 2 – Chemical and technological parameters of mature marsh, depending on the quantity of glucoamylase and concentrate of wort

№	[DS],%	[α-amylase], units of α-amylase ability/g of starch.	[glucoamylase], units glucoamylase ability/g of starch	∑ CO_2, g/200cm³	Acidity, of degrees	Content of non – fermented carbohydrates, g/100cm³		Alcohol content,% vol
						Soluble carbohydrates	Insoluble carbohydrates	
1	18	0.4	5.0	17.75±0.1	0.44±0.1	0.250±0.01	0.080±0.01	10.50±0.01
2	18	0.4	7.5	17.78±0.1	0.44±0.1	0.240±0.01	0.075±0.01	10.51±0.01
3	18	0.4	10.0	18.00±0.1	0.44±0.1	0.230±0.01	0.070±0.01	10.51±0.01
4	27	0.6	5.0	20.30±0.1	0.44±0.1	0.450±0.01	0.100±0.01	13.29±0.01
5	27	0.6	7.5	20.30±0.1	0.44±0.1	0.420±0.01	0.090±0.01	13.35±0.01
6	27	0.6	10.0	20.36±0.1	0.44±0.1	0.410±0.01	0.090±0.01	13.36±0.01
7	30	0.6	5.0	21.50±0.1	0.43±0.1	0.650±0.01	0.110±0.01	15.75±0.01
8	30	0.6	7.5	21.70±0.1	0.43±0.1	0.620±0.01	0.120±0.01	15.78±0.01
9	30	0.6	10.0	21.75±0.1	0.43±0.1	0.610±0.01	0.120±0.01	15.78±0.01

With glucoamylase concentration increased to 10.0 units of glucoamylase ability/g of starch, no noticeable changes in the technological characteristics of the washes were observed.

Since the starch-like material includes not only starch as the main component, but also protein, β-glucans, hemicellulose, gum-substances, and cellulose,

this should be taken into account, especially when fermenting wort of high concentrations.

Processing of high concentration wort raises its viscosity, that is why, to increase the biocatalysis of all grain components, it is necessary to select purpose-oriented multienzymatic systems.

The soluble fraction of non-starch polysaccharides is highly viscous, and the pregelatinisation of starch is much slower. It leads to a decrease in the degree of its hydrolysis by amylolytic enzyme preparations. Besides, when using cellulolytic and proteolytic enzyme preparations, rheological parameters of wort also improve.

In this regard, studies have been carried out to select the compositions of enzyme preparations and their concentrations. When selecting enzyme systems, it was investigated how they affect the techno-chemical characteristics of fermented washes during wort fermentation.

The maize wort fermented had the DS concentrations 18–27%. The maize starch content was 69.0%, the degree of milling dispersion 100% when passing through a sieve with the mesh diameter 1 mm.

A detailed analysis of Table 3 has shown that the concentrations of dissolved carbohydrates and non-dissolved starch tend to decrease when the wort concentration is 18% with increased concentration of cytolytic enzymes. When the cytolytic enzyme concentration was the highest (sample 4), the concentration of alcohol in wash distillates increased by 0.80%, compared with the reference sample. In the samples where a proteolytic enzyme preparation was added, there was an increase in the accumulation of yeast cells, and with the maximum amount of this enzyme, 0.05 units of proteolytic ability/g of raw materials, their concentration increased by almost 6.5%, compared with the reference sample. The addition of cytolytic and proteolytic enzyme preparations in a complex with β-glucanase and xylanase (sample 15) led to a 1.7% increase in the accumulation of ethanol in the washes, compared with the reference sample, and to a decrease in the concentration of dissolved carbohydrates and insoluble starch by almost 33%. With an increase in the wort concentration to 27.0% of DS, the techno-chemical characteristics tended to change in a similar way. The highest concentration of the enzymes under study led to an increase in the accumulation of alcohol in the washes by 1.8%, compared to the reference sample, and the content of soluble carbohydrates in samples 29 and 30 was 0.42 g/100 cm³ of the wash, whereas in all other samples, it was higher by 2–7%, compared to samples 17–25 (Table 3).

Table 3 – Chemical and technological characteristics of fermented wash, depending on the quantitative and qualitative composition of complexes of enzyme preparations and on the wort concentration

№	[DS], %	[α-amylase], 0.4–0.6 units of α-amylase ability/g of starch; [glucoamylase], 7.5 units of glucoamylase ability/g of starch			∑ CO_2, g/200 cm³	Acidity, degrees	Content of dissolved carbohydrates, g/100 cm³	Content of insoluble starch, g/100 cm³	Alcohol content, % vol.	Concentration of yeast cells, mln/cm³
		[protease], units	[cellulase], units	[β-gluca-nase and xylanase], units						
1	18	Reference sample			18.07±0.1	0.45±0.1	0.300±0.01	0.120±0.01	9.50±0.01	155±15
2	18		0.125		18.12±0.1	0.45±0.1	0.291±0.01	0.110±0.01	9.50±0.01	155±15
3	18		0.250		18.20±0.1	0.45±0.1	0.285±0.01	0.100±0.01	9.55±0.01	160±16
4	18		0.350		18.25±0.1	0.45±0,1	0.280±0.01	0.100±0.01	9.58±0.01	164±16
5	18	0.02			18.17±0.1	0.45±0.1	0.290±0.01	0.089±0.01	9.59±0.01	158±15
6	18	0.03			18.23±0.1	0.45±0.1	0.275±0.01	0.084±0.01	9.61±0.01	162±16
7	18	0.05			18.27±0.1	0.45±0.1	0.260±0.01	0.089±0.01	9.64±0.01	165±16
8	18			0.05	18.17±0.1	0,45±0.1	0.300±0,01	0.087±0.01	9.54±0.01	156±15
9	18			0.07	18.23±0.1	0.45±0.1	0.320±0.01	0.087±0.01	9.62±0.01	160±16
10	18			0.10	18.28±0.1	0.40±0.1	0.335±0.01	0.087±0.01	9.64±0.01	163±16
11	18	0.02		0.05	18.39±0.1	0.43±0.1	0.275±0.01	0.100±0.01	9.58±0.01	170±17
12	18	0.03		0.07	18.45±0.1	0.43±0.1	0.264±0.01	0.090±0.01	9.60±0.01	170±17
13	18	0.05		0.10	18.60±0.1	0.43±0.1	0.255±0.01	0.085±0.01	9.60±0.01	172±17
14	18	0.03	0.25	0.07	18.60±0.1	0.42±0.1	0.250±0.01	0.085±0.01	9.65±0.01	175±17
15	18	0.05	0.35	0.10	19.00±0.1	0.45±0.1	0.240±0.01	0.080±0.01	9.67±0.01	175±17
16	27	Reference sample			21.30±0.1	0.44±0.1	0.750±0.01	0.250±0.01	13.10±0.01	180±18
17	27		0.125		21.48±0.1	0.45±0.1	0.750±0.01	0.240±0.01	13.10±0.01	185±18
18	27		0.250		21.50±0.1	0.44±0.1	0.690±0,01	0.220±0.01	13.20±0.01	188±18
19	27		0.350		21.64±0.1	0.44±0.1	0.685±0.01	0.210±0.01	13.20±0.01	190±19
20	27	0.02			21.52±0.1	0.44±0.1	0.540±0.01	0.140±0.01	13.22±0.01	192±19
21	27	0.03			21.61±0,1	0.45±0.1	0.500±0.01	0.140±0.01	13.24±0.01	196±19
22	27	0.05			21.68±0.1	0.45±0.1	0.495±0.01	0.140±0.01	13.28±0.01	199±19
23	27			0.05	21.51±0.1	0.44±0.1	0.580±0.01	0.130±0.01	13.19±0.01	203±20
24	27			0.07	21.60±0.1	0.44±0.1	0.560±0.01	0.130±0.01	13.23±0.01	206±20
25	27			0.10	21.67±0.1	0,44±0,1	0.510±0.01	0.120±0.01	13.27±0.01	207±20
26	27	0.02		0.05	21.75±0.1	0.44±0.1	0.680±0.01	0.200±0.01	13.20±0.01	210±20
27	27	0.03		0.07	21.84±0.1	0.43±0.1	0.650±0.01	0.195±0.01	13.22±0.01	224±20
28	27	0.05		0.10	21.90±0.1	0.43±0.1	0.600±0.01	0.182±0.01	13.30±0.01	224±20
29	27	0.03	0.25	0.07	22.20±0.1	0.43±0.1	0.580±0.01	0.150±0.01	13.31±0.01	224±20
30	27	0.05	0.35	0.10	22.45±0.1	0.42±0.1	0.580±0.01	0.138±0.01	13.34±0.01	200±20

In the samples where the complex enzyme preparation of β-glucanase and xylanase was used, not only an increase in the synthesis of alcohol by 1.2–1.23% was observed, but also an increase in the content of fermentable carbohydrates. This is due to the fact that when using this enzyme preparation, non-starch polysaccharides are hydrolysed, some of which are not fermented by yeast, but react to the anthrone reagent.

Using a complex of β-glucanase and protease, with the right dosage found, ensures high quality of the wort constituents, as well as its effective fermentation and an increase of the alcohol concentration in the washes.

The use of the cytolytic enzyme preparation Laminex 750, at the stage of saccharification and fermentation of the wort, in an amount of 0.250–0.350 units of cytolytic ability/g of raw materials, contributed to an increase in the alcohol content in the washes by 0.3–1.4%, depending on the wort concentration, compared with the reference sample. It also resulted in reducing the concentration of fermentable carbohydrates and insoluble starch by 14–23%. Thus, on the basis of experimental studies, it has been found that the use of a complex of enzyme preparations – amilolytic (Amylex 4T), saccharifying (Diazyme TGA), proteolytic (Alphalase AFP), cytolytic (Lamenex 750), and complex enzyme preparations of β-glucanase and xylanase (Deltazyme VR XL) in various combinations of their concentrations – contributed to intensification of wort fermentation and an increase in the accumulation of the target product, ethanol, by 0.8–1.4%, depending on the wort concentration. The largest amount of ethanol was accumulated at the maximum dosage of additional enzyme preparations.

Conclusions

It is recommended to use the following concentrations of enzyme preparations per an activity unit: Amylex 4T (the source of α-amylase) – 0.4–0.6 units of α-amylase ability/g of starch, which is optimal for the DS concentration 18–27%, and for 30% of DS, it is better to use 0.6 units of α-amylase ability/g of starch; Diazyme TGA (the source of glucoamylase) – 7.5 units of glucoamylase ability/g of starch; Laminex 750 (the source of the cytolytic enzyme) – 0.35 units of cytolytic ability/g of raw materials, Alphalase AFP (the source of the proteolytic enzyme) – 0.05 units of proteolytic ability/g of raw materials; Deltazyme VR XL (the source of β-glucanase and xylanase) – 0.05 units of β-glucose ability/g of raw materials. The growth of the specified parameters during fermentation of wort of high concentrations can be taken into account in the complex processing of carbohydrate-containing raw materials with further selling the distillers' grains to farms and production of mixed fodders.

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