1. Introduction

Analysis of data from the brewing industry shows that despite difficult economic conditions, quality beer is rapidly gaining popularity in recent years. To maintain the position of their brands in the market, producers are expanding their range of specialty beers. The emergence of an innovative product revives the beer market and opens up new prospects. Today the brewing industry is one of the most attractive investment sectors of the economy. Beer is one of the most commonly consumed drinks and brewing companies produce a fairly wide range of beer, each of which finds its own consumer [1].

However, this industry is still not provided in sufficient quantities with quality raw materials, in particular, malting barley. Processing non-brewed barley with a high protein content (above 12 %) and low starch content and extractiveness into beer from an economic point of view is unprofitable, and from the viewpoint of quality is undesirable [2, 3].

The most important directions in solving this problem should be recognized as the improvement and development of new resource-saving technologies of malt and beer using non-traditional types of raw materials [4, 5].

The shortage of malting barley – the main raw material for the brewing industry – makes the use of non-traditional types of grain a topical issue. It is known that for the production of beer, barley, wheat, rye are processed in a large degree, as well as malt is obtained from the grain culture. In addition, traditional cereals, such as triticale, amaranth, sor-
ghum, buckwheat, oats, etc. have been used extensively for fodder purposes for some time. There are many advantages to using new raw materials in brewing. They make it possible to produce new beer with an interesting taste and aroma. In addition, the use of certain raw materials can reduce production costs or improve various quality characteristics such as taste, color or turbidity. However, very often new raw materials, besides barley malt, cause difficulties in brewing, for example, too long saccharification or filtration time, too low level of wort extract and, therefore, the alcohol content in the beer, the need to add exogenous enzymes, etc. Thus, it is advisable to continue studying the malting properties of non-traditional cereals for the production of brewing malt.

2. Literature review and problem statement

Nowadays, non-traditional crops are gaining popularity in the brewing industry. This is happening both with the aim of expanding the range of products and obtaining “new tastes”, as it is caused by the need to replace imported raw materials with domestic ones and reduce the cost of production [6].

In the research [7], the data on the improvement of production technology of oatmeal, suitable for brewing are given. Do not look at the fact that oat grains are distinguished by increased food value, have a high content of non-starch polysaccharides, which complicates the process of hydrolysis of starch. Suggested regimens of fertilization provide the introduction of enzyme preparations already at the stage of grain fermentation. From the point of view of economic efficiency, this technology is not widely used in the production of brewed malt.

The works [8, 9] present the results of research aimed at the production of brewing malt from grain sorghum. It has been shown that higher temperatures were used in the production of malt, both during soaking (30 °C) and during germination of grain (25 °C) than in the production of barley malt. This is due to the thermophily of the culture and the low content of β-amylase. Also, a characteristic feature of sorghum, which causes certain difficulties when using this grain in brewing, is its high loss during malting – up to 7 % [10, 11].

The authors [12] investigated the possibility of using buckwheat malt in brewing. The data of studies of the dynamics of amylolytic activity and moisture accumulation under different modes of malting in buckwheat grain, differing in protein and starch content, are presented. It was found that under conditions of periodic irrigation with an uncontrolled accumulation of moisture (up to 58 %), the maximum amylolytic activity of freshly germinated malt is about 300 units. Amylolytic activity was noted after three days of germination at a temperature of 15 °C in both low-protein and high-protein varieties. However, the use of buckwheat malt in the production of beer on a large scale is not advisable, since due to the high content of phenolic compounds, beer varieties with the use of buckwheat malt will have low colloidal stability, therefore, the production of such a drink is possible only at breweries of small capacity, where the sales period of products is short [13].

In [14], the potential of new raw material – tritordeum for beer production is studied. The results of grain analysis did not show significant differences between tritordeum and barley malt in terms of physicochemical characteristics, while in the tritordeum sample, less glassy grains and better gradation were determined when studying the absolute mass. However, there are objective difficulties in the use of tritordeum malt in the production of beer, associated with an increased protein content, which can lead to colloidal instability of the final product during storage.

Among the alternative crops, triticale should be noted as the most promising type of grain raw material [15]. Since triticale is superior to barley in terms of the total amount of extract and other chemical indicators, this indicates that the use of this crop as a raw material for the production of brewing malt is a promising direction in brewing [16, 17].

The first artificially created grain has been discussed for some time [18], but the possibility of using triticale grain for the production of malt and its use in brewing was investigated only by a number of scientists from the countries of near and far abroad [19–23].

Development of technological parameters of malting of Kazakhstan varieties of triticale grain, determination of its physicochemical, analytical characteristics will make it possible to create new raw materials for beer production [24].

An important indicator for the quality of freshly sprouted malt is its enzymatic activity. One of the main requirements for brewing malt is its fast self-saccharification, determined by the amylolytic capacity of the malt, which is expressed by the amount of maltose (in grams) formed from starch under the action of enzymes per 100 g of malt [25].

The amount of amylolytic activity changes with the content of amylolytic enzymes and depends on many factors (variety, growing conditions, climatic conditions). The process of malting has the greatest influence on this parameter, as a result of which these enzymes are activated [26].

By selecting the modes of malting, one can achieve a significant change in malt parameters and a significant economic effect. However, when changing the modes of malting, it is necessary to control the amylolytic activity of malt, which is one of the critical indicators of the process.

All this suggests that it is advisable to conduct research on the development of optimal modes of malting triticale grain and the study of the accumulation of α- and β-amylase of brewing malt from triticale grain in order to replace barley malt with it in beer production, which will expand the raw material base and the range of products.

3. The aim and objectives of the study

The aim of the scientific research is to study the amylolytic activity of malt from triticale grains, according to selected modes of malting. This will make it possible to expand the raw material base and range of the brewing industry by introducing a fundamentally new technological scheme for the production of brewing malt from triticale grain, reducing technological costs as a result of reducing the production cycle time, both at the stages of steeping and germinating malt, and due to higher enzymatic activity of unconventional triticale malt.

To achieve the aim, the following objectives were set:

– selection of the optimal method for soaking and germinating triticale grain;
– study of the accumulation of α-, and β-amylase in triticale grain malt according to the selected parameters of malting;
– statistical processing of the obtained data.
4. Materials and methods of research

The objects of research are the Kazakh grain variety triticale – Balausa 8, experimentally developed and submitted for research by the Kazakh Research Institute of Agriculture and Plant Production LLP (Almaty region, Kazakhstan).

Experimental studies on the quality indicators of freshly sprouted malt from triticale grain varieties “Balausa 8” were carried out in the research laboratory for undergraduates and doctoral PhD students at the Department of Food Technology, University of Natural Resources and Applied Sciences “BOKU”, Vienna (Austria), as well as in the research laboratory “Food Safety” of the Almaty Technological University.

Determination of the activity of amylolytic enzymes was carried out according to the EBC 4.13 method using a Malt Amylase Assay Kit from Megazyme Kit (Ireland).

The α-amylase content in malt was determined using the Malt Amylase Assay Kit by the Ceralpha method. During the hydrolysis of the oligosaccharide by endoactive α-amylase, the excess amount of α-glucosidase present in the mixture gives instant and quantitative hydrolysis of the p-nitrophenylmalto-saccharide fragment to glucose and free p-nitrophenol.

The β-amylase content in malt was also determined using the Malt Amylase Assay Kit using the Betamyl – 3 method. This test reagent uses high-purity β-glucosidase and p-nitrophenyl – β-D-maltotrioside (PNPG3). Thermolabile β-glucosidase has no effect on the native substrate due to its inability to cleave α-linked D-glucosyl residues. During hydrolysis of PNPG3 to maltose and p-nitrophenyl – β-D-glucose by β-amylase, p-nitrophenol remains in excess in the substrate mixture. Thus, the rate of release of p-nitrophenol relates directly to the rate of release of maltose by β-amylase.

0.25 g of the crushed test sample of malt was weighed into a polypropylene test tube and 2.5 ml of buffer solution “Buffer A” diluted with cysteine HCl (0.88 g/50 ml) was added to obtain an amylase extract, then the contents were thoroughly shaken using a Vortex stirrer for 5 sec. Thus, the sample was shaken every 10 minutes for an hour. After extraction, the samples were centrifuged at 4,000 rpm for 10 min. 0.1 ml of the obtained filtrate was poured into a Vortex mixer and incubated again for 10 minutes. After 10 minutes, 1.5 ml of Tris stop Reagent with high pH was added. The resulting solution (A<sub>400</sub> (sample)) was transferred to a spectrophotometer cuvette and read at 400 nm against a control sample. The control sample (A<sub>400</sub> (control)) is a blank sample, prepared by adding 1.5 ml of Tris stop reagent to 0.1 ml of “Substrate A” and 0.1 ml of “Extract 2” (in that order).

The calculation of the α-amylase activity was carried out according to the formula (2):

\[
\alpha-\text{amylase:}[U/\text{g}] = \frac{(A_{400}\text{ (sample)} - A_{400}\text{ (control)})}{3.156}.
\]

For the objectivity of judgment on the degree of reliability of the results obtained, their mathematical processing was carried out using a full factorial experiment (PFE 22) using a central compositional rotatable uniform planning.

Statistical analysis of the regression equations was carried out using the STATISTICA 10.0 and Microsoft Excel software package included in the experiment planning matrix according to the chosen method.

5. Results of the research on the amylolytic activity of malt from triticale grains, according to selected modes of malting

5.1. Selection of the optimal modes of triticale malting

In the production of malt, conditions are artificially created in order to change the properties of the grain, leading to the accumulation of the maximum possible amount of enzymes and achievement of a certain biochemical composition. The necessary conditions arise during soaking and germination. The intensity of the formation and accumulation of enzymes is determined by the rate and type of carbohydrate desimilation process, which is a series of redox reactions. To carry out these reactions, oxygen, free water, and a certain temperature are required [27].

To determine the optimal method of soaking (up to a moisture content of 40%, 42% and 44%) and germination (3, 4 and 5 days) of triticale grains, three main temperature regimes (14°C, 16°C and 18°C) were selected in Fig. 1.
According to the data in Fig. 1, soaking to a humidity of 40%, 42% and 44% was carried out at temperatures of 14°C, 16°C and 18°C, in chambers with a relative humidity of 98%.

Malting, for each selected mode, was carried out three times, the amount of grain in each basket is 300 g. Thus, by the end of the experimental work, 27 samples of freshly sprouted malt prepared according to 27 possible parameters of malting were studied.

Drying of freshly prepared malt was carried out in several stages, regardless of the chosen parameters of soaking and germination.

Upon completion of the germination process, the temperature was gradually increased to 70°C. In general, the malt drying period is 33.3 hours, where the moisture content gradually decreased from 40–44% to 5–6%.

The choice of a milder and more gentle drying mode, in contrast to the classic drying mode of barley malt, is explained by the peculiarity of the anatomical structure of triticale grain, namely, the absence of chaff.

After drying, freshly prepared triticale malt was cooled for 24 h at room temperature, and the shoots were removed. After a week of maturation, at a temperature of 4°C, the amylolytic activity of the finished malt was determined.

5.2. Accumulation of amylolytic enzymes during the malting process

The duration of the malt preparation process and its quality indicators are determined by the rate of accumulation of hydrolytic enzymes. According to the selected modes of malting (described in Section 5.1), samples of germinating triticale were taken daily and the amylolytic (α- and β-amylase) activity of enzymes was determined.

Experimental data on the influence of the degree of soaking and temperature regimes of germination on the dynamics of accumulation of α-amylase of triticale in the test sample is shown in Fig. 2.

According to the data in Fig. 2, the activity of α-amylase sharply increases in all selected modes on the fourth day of growth, then the increase is insignificant. Sprouting at 18°C to obtain the desired malt properties can be considered ineffective due to the appearance of a smear consistency. Germination at this temperature regime is characterized by uniform grain growth, but a slowdown in the accumulation of enzymes. Thus, the activity of α-amylases reaches only 150 units on the 5th day of growth.

During germination at a temperature of 14°C, an increase in the process of accumulation of enzymatic activity is observed, but the most intense accumulation of enzymes occurs with a degree of soaking of 44% and germination at 16°C and reaches 217.99 units on the fifth day of growth. The maximum value of activity at a given temperature is on average 7% higher than at a temperature of 14°C and by 38% than at a temperature of 18°C. Probably, germination according to this mode causes an accelerated synthesis of α-amylase, due to an increase in the rate of diffusion of gibberellins-like substances to the cells of the aleurone layer.

The experimental data are described by a linear equation with a sufficiently high accuracy $R^2 = 0.983$.

Data on the influence of the degree of soaking and temperature regimes of germination on the dynamics of accumulation of β-amylase of triticale in the test sample are presented in Fig. 3.

According to the data in Fig. 3, during triticale malting according to the selected parameters, the β-amylase activity increases uniformly and increases with an increase in the degree of soaking, reaching the highest values on the third day of growing, and then the increase is insignificant.

During germination at a temperature of 14°C, the accumulation of the smallest amount of β-amylase (23.3 units) is observed. This is explained by the fact that the activity of β-amylase of triticale grain, which is in an inactive form, blocked by protein substances, largely depends on the amount of protein and the activity of proteolytic enzymes, which breaks down the repressor protein.
The maximum value of the activity of $\beta$-amylase of triticale is observed during germination at 16 °C (33.32 units), which is 18 % higher than at 14 °C and 6 % higher than at 18 °C. The experimental data are described by a linear equation with a sufficiently high accuracy $R^2=0.97$.

5.3. Mathematics for data processing
The data were processed mathematically to confirm the reliability of the obtained results of the study to determine the accumulation of amylolytic enzymes in malt from triticale grain.

The parameters of the regression equations are estimated in STATISTICA 10. The following factors were used as the main factors influencing amylolytic activity during germination: $X_1$ – degree of soaking, %; $X_2$ – temperature, °C; $X_3$ – duration of germination, days. The criteria for assessing the influence of factors on the activity of hydrolytic enzymes were: $Y_1$ – $\alpha$-amylase activity, units; $Y_2$ – $\beta$-amylase activity, units. The limits of change in factors are presented in Table 1.

For the main level, the germination mode is selected, which ensures the normal course of the accumulation of hydrolytic groups of enzymes.

When processing the results of experimental data, the following statistical criteria were used: Cochran’s criterion – to check the homogeneity of the variance; Student’s test – to check the significance of the coefficients of regression equations; Fisher’s criterion – as the adequacy of the equations.

Regression equations of the second degree are obtained, describing the change in the activity of hydrolytic groups of enzymes in malt from triticale under the influence of the factors under study:

$$Y_1 = -331.9063 + 13.8108X_1 - 10.2225X_2 + 15.369X_3,$$

$$Y_2 = 16.2196 + 0.5961X_1 - 0.9545X_2 + 0.4185X_3.$$

The analysis of the regression equation makes it possible to single out the factors that have the greatest influence on the activity of hydrolytic enzymes of triticale during malting.

The activity of $\alpha$-amylase (3) is more influenced by $-0.5961X_1$ (degree of soaking, %) and 15.369 $X_3$ (duration of germination, days), less influence is exerted by $13.8108X_2$ (degree of soaking, %). With linear terms, the regression coefficients are positive, which indicates that all technological factors have a “positive” effect on the accumulation of $\alpha$-amylase.

The correlation coefficients of the activity of $\beta$-amylase (2) is also more influenced by $-0.9545X_2$ (temperature, °C) and 0.4185 $X_3$ (germination duration, days), a lesser effect is exerted by 0.5961 $X_1$ (degree of soaking, %). However, the factors have a “negative” effect, which will lead to a decrease in the accumulation of $\beta$-amylase, with an increase in the values of technological factors during malting.

The regression equation took the form of a second-order equation, which made it possible to display the response surface of the function geometrically.

The possibility of obtaining lines of levels of dependence of enzymatic activity on factors influencing germination at a temperature of 16 °C gave the design of the surface of the response of the function to the plane, Fig. 4, 5.

From Fig. 4, 5, it is seen that increasing the degree of soaking and the duration of germination of triticale increases the activity of enzymes.

Under the studied conditions of germination, the accumulation of $\alpha$-amylase in triticale grains increases rather sharply until the fourth day of growing, then the activity of $\alpha$-amylase increases more evenly (Fig. 4). It is known that an increase in the degree of soaking, temperature and duration of germination activates the synthesis of this enzyme.
Fig. 4. Change in α-amylase activity during germination of malt from triticale grain at a temperature of 16 °C

Fig. 5. Change in β-amylase activity during germination of malt from triticale grain at a temperature of 16 °C
The activity of β-amylase increases fairly evenly during malting at a temperature of 16 °C, and increases with increasing moisture content, reaching the highest values on the fifth day of growing (Fig. 5). In general, an increase in the degree of soaking up to 44 % and the influence of technological modes of malting on the activity of β-amylase are not great.

The study of the dynamics of the accumulation of amylolytic enzymes in triticale grain in the process of malting according to the selected modes showed that the most favorable degree of soaking is 44 %, the duration of malting is 5 days, at a temperature of soaking and germination of 16 °C.

### 6. Discussion of experimental results

According to the data of experimental studies, the optimal mode of malting (soaking to a moisture content of 44 %, germination for 5 days at a temperature of 16 °C) of triticale was determined, which made it possible to ensure the maximum accumulation of amylolytic enzymes.

Soaking to higher humidity values is inappropriate, due to the fact that triticale, a filmless grain that is sensitive to squeezing, cakes heavily and excessive water absorption can occur with all the disadvantages of intramolecular respiration, uneven germination and obtaining a smeared endosperm consistency upon dissolution.

The temperature of the water used during soaking should not be too high, so as not to adversely affect the vital activity of the grain, especially the embryo. In water at a higher temperature, the solubility of oxygen is less than at a lower one. At the same time, at a temperature of 18–20 °C, the bacterial microflora located on the surface of the grain develops intensively and consumes a significant amount of oxygen, which can lead to a lack of oxygen for the respiration of the embryo.

The dynamics of water absorption by triticale grain at a temperature of 14 °C is active, but the most intensive accumulation of moisture is observed when soaking at a temperature of 18 °C. In [28], a warm mode of steeping triticale grain is used, however, when soaking in this mode, early pecking of the grain is observed, which leads to the loss of dry matter.

It was found that the optimal temperature for soaking triticale grains of the “Balausa 8” variety should be considered 16 °C. At the selected temperature regime, the degree of triticale soaking is achieved after 18 hours of soaking, which reduces the process by 2–2.5 times compared to barley; the optimal mode of malting for 5 days at a temperature of 16 °C, which made it possible to ensure the maximum accumulation of hydrolytic enzymes.

Soaking to higher humidity values is inappropriate, due to the fact that triticale, a filmless grain that is sensitive to squeezing, cakes heavily and excessive water absorption can occur with all the disadvantages of intramolecular respiration, uneven germination and obtaining a smeared endosperm consistency upon dissolution.

The optimal mode of triticale malting was experimentally determined (soaking to a moisture content of 44 %, germination for 5 days at a temperature of 16 °C), which made it possible to ensure the maximum accumulation of hydrolytic enzymes. Accelerated synthesis of amylases (217.99 units) occurs due to an increase in the rate of diffusion of gibberellin-like substances to the cells of the aleurone layer. The obtained regression equations made it possible to calculate the activity of the indicated malt enzymes depending on the technological factors of the process.

The proposed modes of obtaining malt from triticale grain will make it possible to intensify the production process by reducing the duration of the soaking (24 hours) and germination (5 days) processes in comparison with the traditional technology for preparing barley malt.

The use of triticale malt in brewing will improve the raw material base of the brewing industry, which leads to an expansion of the range of products, reduced product costs due to the higher extractability of triticale malt compared to barley. Replacing the malt barley malt with triticale will save expensive raw materials. However, the disadvantage of using triticale malt in beer production may be due to the content of mucous substances – pentosans, inherited from parents, which can lead to increased viscosity of the wort. In this regard, it is necessary to solve further issues on the study of the accumulation and changes in the carbohydrate composition of triticale malt, carry out mathematical calculations to select the optimal amount of grist in the mash.

The main difficulties in these studies are the small sown areas of this crop, but every year this problem is solved. In the future, triticale will take one of the leading places among grain crops. Also, the use of triticale malt as the main raw material in the production of beer will allow the mashing process to be carried out without enzyme preparations due to the high α- and β-amylase activity in it. The combined use of barley malting and triticale malt will increase the yield of marketable beer, improve its quality and reduce the cost of production.

### 7. Conclusions

1. The optimal modes of malting were selected: the temperature of soaking of triticale grains of the “Balausa 8” variety should be considered 16 °C. At the selected temperature, the degree of triticale soaking is achieved after 18 hours of soaking, which reduces the process by 2–2.5 times compared to barley; the optimal mode of malting for 5 days at a temperature of 16 °C, which made it possible to ensure the maximum accumulation of hydrolytic enzymes.

2. The accumulation of amylolytic enzymes has been studied: the most intensive accumulation of α-amylase enzymes occurs at a degree of soaking of 44 % and germination at 16 °C and reaches 217.99 units on the fifth day of growth. The maximum value of activity at a given temperature is on average 7 % higher than at a temperature of 14 °C and by 38 % than at a temperature of 18 °C; the maximum value of the activity of β-amylase of triticale is observed during germination at 16 °C (33.32 units), which is 18 % higher than at 14 °C and 6 % higher than at 18 °C.

3. Statistical processing of the obtained experimental data was carried out using the method of central compositional rotatable uniform planning of the experiment in STATISTICA 10. As a result, the reliability of the previously obtained experimental results of the study on the accumulation of amylolytic enzymes in malt from triticale grain was proved.

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