Development of technology for the oat wort's production using over 50% husked oat malt with the application of enzymatic preparations

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Abstract. Oat malt is of considerable interest for the development of new functional beverages due to its high dietary fiber content. In order to harness the biopotential of non-starch polysaccharides, which are present in the cell walls of the aleurone layer and endosperm, it is also essential to enhance the extract yield from oat malt exhibiting low extractability (42.8%). This study investigates the effects of enzyme preparations (EP) with high glucoamylase, α-amylase, and cytolytic activity, as well as the endogenous enzymes of malt, on the physicochemical properties of wort quality when utilizing varying amounts of oat malt in the mash. The findings indicate that the incorporation of only enzyme preparations during the mashing of oat malt is insufficient to address the challenges associated with increasing the economic viability of utilizing this raw material. It was only after substituting 50% of oat malt with barley malt and concurrently applying EP that it became possible to obtain wort with a high extract yield, elevated viscosity (1.809 mPa·s), and a turbidity of 5.2 EBC units, which was more than 20 times lower than that of wort produced solely from oat malt.

1 Introduction

In recent years, there has been a growing interest in studying oat malt as a potential raw material for the production of not only food products but also plant-based beverages [1-6]. Oats, due to their rich chemical composition and beneficial properties, represent an attractive alternative to traditional cereal crops [7, 8]. Currently, the oat wort production is regulated by a number of technical and regulatory documents ensuring the standardization of processes and the products quality. One such document is GOST R 70650-2023 "Plant-Based Beverages (from Grains, Nuts, and Coconut). General Technical Specifications" [9].

Oat malt significantly impacts the quality of brewing products, as well as the functional properties of the finished beverage:• it enhances the richness of the flavor and aroma profile of beer, which is particularly valued in styles such as oatmeal stouts and ales;

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• it increases foam stability, making the beer visually appealing and improving the sensory experience during consumption.

It is important to note that beverages produced from both oats and oat malt contain significantly higher levels of soluble dietary fibers (beta-glucans, arabinoxylans, gums, and pectins) compared to those made from other grains. The significance of these compounds for human health cannot be overstated, as they contribute to the improvement of cardiovascular system function by lowering blood glucose and cholesterol levels [10-12].

This is why oat malt is a relevant ingredient for the plant-based fermented beverages production. However, the use of oat malt in wort production for both alcoholic and non-alcoholic products faces several technological challenges, among which viscosity and extractability are key issues. These indicators reduce the efficiency of the wort filtration process, increasing losses in the brewing department. In this regard, the enzyme preparations application aimed at optimizing these parameters becomes essential for improving wort yield.

When using oat malt, it is necessary to maintain a balance in the malt blend to avoid negative effects on the filtration process (such as increased product viscosity and reduced filterability). To optimize these parameters during the mashing process, enzyme preparations hydrolyzing arabinoxylans and glucans into low-molecular-weight carbohydrates are employed [6, 13, 14, 15].

This article is dedicated to the comprehensive analysis of the various enzyme preparations impact on the process of obtaining wort from oat malt and its main characteristics, which opens up new opportunities for optimizing production processes in the brewing industry [16,17].

2 The purpose and objectives of the study

The aim of this study is to create a technology for the production of fermented beverages based on extract from oat malt. To achieve this objective, the following tasks were addressed:

• Justify the selection of mashing conditions for the malts;

• Determine the physicochemical characteristics, such as filterability, viscosity, and extractability of 100% oat malt during mashing;

• Investigate the impact of the ratio of oat malt to barley malt in the grain bill on the physicochemical parameters of the wort.

• Examine the enzymatic activity of enzyme preparations with the aim of optimizing the mashing process.

3 Materials and methods of research

The materials of the study were: oat and barley malt, meet the requirements of the regulatory document. Oat malt must comply with TU 11.06.10-007-13185567-2020 - for oat malt and GOST 29294-2021 - for barley malt [18].

To study the biopotential of oat malt, exogenous enzyme preparations of foreign origin (Denmark) were used. The characteristics of the enzyme preparations (EP), the physicochemical conditions for the maximum activity manifestation and the recommended dosages for obtaining beer wort from barley malt are given in Table 1.

Table 1. Characteristics of enzyme preparations

Enzyme preparation	Units of activity	Optimal conditions for the manifestation of 80-100% activity		Recommended dosage, g EP/kg malt
		Temperature [°C]	pH	
Glucoamylase	min. 350 G AU/g	62-73	3,6-5,3	0,5-10,0
Alpha-amylase	min. 13 775 A AU/g	75-90	4,5-8,0	0,05-0,20
Combined EP: β-glucanase (90%); xylanase (10%)	min. 9 090 B BU/g min. 1380 X BU/g	55-78	5,3-6,2	0,05-0,40

Glucoamylase is a saccharifying enzyme preparation derived from the culture broth of the fungus Aspergillus niger. The broad range of recommended dosages allows for the selection of a specific dosage to obtain wort with desired characteristics (sugar spectrum and final degree of fermentation). The producer of thermostable alpha-amylase is Bacillus licheniformis. The combined enzyme preparation is obtained through the cultivation of Trichoderma reesei and Bacillus subtilis. The preparation's action is aimed at the β-glucans' and arabinoxylans' hydrolysis, which are components of the cell walls of the endosperm and the aleurone layer.

3.1 Methods for Analyzing Malt and Wort

The quality indicators of barley and oat malt were evaluated according to the European Brewery Convention (Analytica-EBC. Method 4.5.1) [19]. The moisture content of barley and oat malt was determined using the Halogen Moisture Analyzer HR73 (Mettler Toledo) at a temperature of 105°C by the method of drying to a constant weight (Analytica-EBC. Method 4.2). In the congress and experimental wort, the following analyses were performed:

• the mass fraction of dry matter, real extract, and real degree of fermentation (RDF) were analyzed using the Alcolyzer automatic analyzer for wort and beer, Anton Paar DMA 4500 (Analytica-EBC. Method 8.3; GOST 12787-2021);

• viscosity was measured using the AND SV-10 vibrating viscometer (Analytica-EBC. Method 8.4);

• the concentration of β-glucan in the wort was determined by spectrophotometric method (Analytica-EBC. Method 4.16.3) using the Enzytec™ Color GlucaTest® test system on a UV-2501 PC spectrophotometer (Shimadzu) at a wavelength of 550 nm;

• diastatic power was determined by the Windisch–Kolbach method (WK; Analytica-EBC. Method 4.12.1);

• the filterability of the wort was assessed based on the volume of wort filtered within 30 minutes. Measurements were conducted in a 250 ml graduated cylinder with a scale interval of 2 ml;

• the turbidity of the wort was determined by the nephelometric method after filtration through a paper filter with the addition of diatomaceous earth on the LabScat 2 turbidity meter (Sigrist-Photometer AG). Turbidity measurements were taken at an angle of 90° with a wavelength of 650 nm.

3.2 Methods of Experiments

3.2.1. Raw Material Characteristics.

Before starting the mashing process [20], the main physicochemical parameters of the malts were studied. Analyses were conducted to determine moisture content, extractability (dry matter), viscosity, β-glucan content, and diastatic power. The physicochemical characteristics of the malts used in the experiments are presented in Table 2.

Table 2. Physicochemical Characteristics of Malts

Name of the Indicator	Results of the Tests		
	Oat Malt	Barley Malt	Requirements
Moisture [%]	6,9±0,2	4,1±0,2	4,5-5,5
Extractivity [%, d.m.]	42,8±0,5	81,9±0,3	>81
Viscosity [mPa*s]	1,81±0,01	1,53±0,01	1,53-1,56
β-Glucan [mg/L]	797±9,8	148±7,7	0-150
Diastatic force [units/g]	11,0±1,5	282±1,2	>250

The results are presented as the mean value from three or more independent experiments. The standard notation follows the symbol ±.

3.2.2. Study of the influence of oat malt on the techno-chemical indicators of wort quality.

Oat and barley malts were ground using a laboratory disc mill DLFU (Bühler). The distance between the discs was set at 0.2 mm. The milling process was conducted immediately prior to mashing. The mashing process was conducted in accordance with the temperature profile presented in Figure 1.

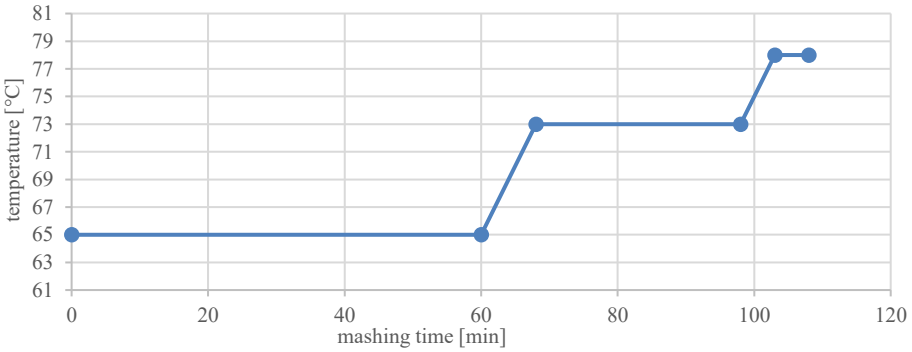

Fig. 1. Optimal mashing regime for oat malt.

The choice of the initial mashing temperature is explained by the fact that oat malt contains a high amount of β-glucan, which significantly increases the viscosity of the wort and thereby reduces the extract yield. To prevent the action of the enzyme β-glucanase, which releases β-glucan from the cell walls of the endosperm and aleurone layer cells, the mashing temperature should be above the optimal range for this enzyme's activity, i.e., above 65°C. At this temperature, amylolytic enzymes (α- and β-amylases) are simultaneously active. To increase the maltose's amount in the extract from the malt (mash), the duration of the rest at 65°C was set to 60 minutes. Subsequently, the temperature was raised at a rate of 1°C/min to

72-73°C, at which α-amylase from the malt acts, performing the hydrolysis of starch to produce dextrins. The duration of this rest was 30 minutes. Enzyme inactivation was carried out at a temperature of 78°C. The mash-to-water ratio was 1:3.5. Enzyme preparations were added at the beginning of the mashing process at 65°C.

The hot mash was filtered using FiFo MN 614 ¼ filter paper with a diameter of 320 mm (Macherey-Nagel), and after cooling to 20°C, the wort was analyzed.

3.2.3. The study of the influence of enzyme preparations on the quality indicators of wort produced from 100% oat malt.

Experiments were conducted using the method of multifactorial analysis. The experiment was designed using a two-factor, two-level factorial design matrix (Table 3), which allowed for the assessment of the interaction between the enzymes and their impact on the final product. The factors were the concentrations of the enzymes – glucoamylase (X1) and α-amylase (X2), while the optimization parameter was the yield of wort during the mashing process. Two series of experiments were conducted. In the first series, the concentration of the combined enzyme preparation with cytolytic activity was 0.2 g/kg of malt, while in the second case, it was 0.5 g/kg of malt.

Table 3. Planning matrix for two factors at two levels

№ опыта	Value of factors in coded units		Value of factors [g/kg]	
	Glucoamylase (X1)	α- Amylase (X2)	Glucoamylase (X1)	α-Amylase (X2)
1	-	-	*0,7*	*1,0*
2	+	-	*2,1*	*1,0*
3	-	+	*0,7*	*2,4*
4	+	+	*2,1*	*2,4*

At the second stage of the research, a comparison was made between the application of maximum and minimum dosages of enzyme preparations. The maximum dosage included: glucoamylase at a concentration of 2.1 g/kg of malt, alpha-amylase at 2.4 g/kg of malt, and a combined enzyme preparation (CEP) at 0.5 g/kg of malt. The minimum dosage consisted of glucoamylase at 0.7 g/kg of malt, alpha-amylase at 1.0 g/kg of malt, and a combined CEP at 0.2 g/kg of malt. The study was conducted with varying proportions of oat malt mixed with type A barley malt in ratios of 10/90, 20/80, 30/70, 40/60, and 50/50, respectively.

3.3. Processing of research results

The results are presented as the mean value of three or more independent experiments. The standard notation follows the symbol ±. The data were analyzed using one-way analysis of variance (ANOVA) [21]. Calculations were performed at a significance level of $p = 0.05$.

4 Results and Discussion

In Tables 4 and 5, the results of the physicochemical parameters' analysis of 100% oat wort obtained using various enzyme preparations are presented. As a statistical processing's result

of the experimental data and the exclusion of non-significant regression coefficients, regression equations (1) and (2) were obtained.

Table 4. Physico-chemical characteristics of 100% oat wort using a combined enzyme preparation (0.2 g/kg of malt)

Experiment No.	Factor values [g/kg of malt]		Filtrate volume [ml]
	Glucoamylase (X1)	α- Amylase (X2)	
1	0,7	1,0	49±10
2	2,1	1,0	41±13
3	0,7	2,4	72±19
4	2,1	2,4	83±14

Table 5. Physico-chemical characteristics of 100% oat wort using a combined enzyme preparation (0,5 g/kg of malt)

Experiment No.	Factor values [g/kg of malt]		Filtrate volume [ml]
	Glucoamylase (X1)	α- Amylase (X2)	
1	0,7	1,0	41±15
2	2,1	1,0	78±20
3	0,7	2,4	52±17
4	2,1	2,4	72±19

Based on the conducted experiments, the following conclusions were drawn. Firstly, an increase in the amount of enzymes in the mash positively affects the volume of the resulting filtrate (see Tables 3 and 4); however, even in the optimal scenario (variant 4), this volume remains significantly lower than what is typically observed in mashes made from barley malt. Secondly, a 2.5-fold increase in the dosage of the combined preparation containing cytolytic enzymes not only failed to enhance the yield of the filtrate but, on the contrary, led to a reduction in its output.

Based on the research data presented in Table 4, the following regression equation (1) has been obtained:

$$Y_1=12{,}0325-0{,}4525*X_1+0{,}3675*X_2, \quad (1)$$

where: Y_1— volume of the filtrate (ml);

X_1 — dosage of glucoamylase (g/kg of malt);

X_2 — dosage of α-amylase (g/kg of malt).

Based on the research data presented in Table 5, the following regression equation (2) has been obtained:

$$Y2=11{,}92+0{,}225*X1-0.045*X2, \quad (2)$$

where Y_1— volume of the filtrate (ml);

X_1 — dosage of glucoamylase (g/kg of malt);

X_2 — dosage of α-amylase (g/kg of malt).

Based on the data obtained from two series of experiments, it can be concluded that enzyme preparations do not provide an adequate level of hydrolysis of the components of oat malt. However, increasing their dosage is not an economically viable option. Therefore, it is necessary to utilize the enzymatic potential of barley malt. To this end, a series of experiments was conducted in which the proportion of oat malt was varied (from 90% to 50%) in barley-oat mashes. The consumption of enzyme preparations calculated per total malt (oat and barley) in the first variant was maximal: glucoamylase – 2.1 g/kg of malt, alpha-amylase – 2.4 g/kg of malt, combined enzyme preparation – 0.5 g/kg. In the second variant, the consumption was minimal: glucoamylase – 0.7 g/kg of malt, alpha-amylase – 1.0 g/kg of malt, combined enzyme preparation – 0.2 g/kg. The main physicochemical properties of the wort produced at different ratios of oat and barley malts are presented in Figure 2 and Tables 6 and 7.

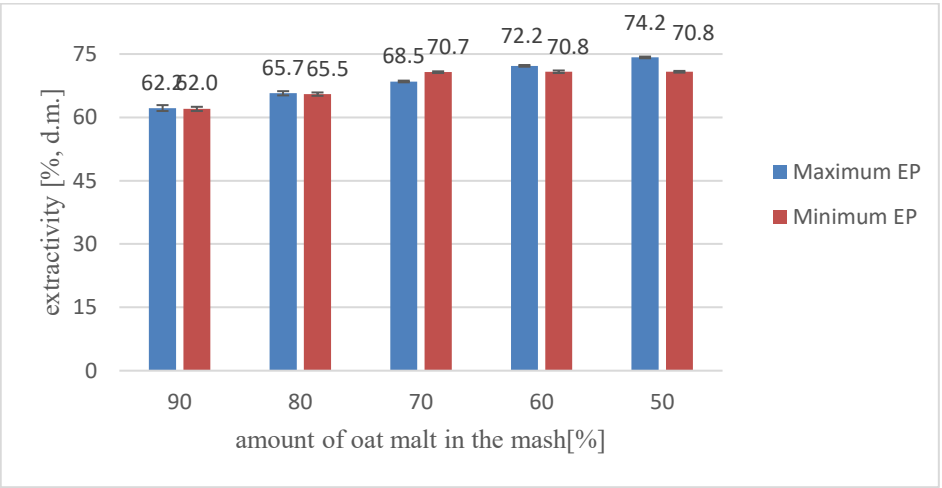

Fig. 2. The extractivity (D.M.) of the wort with different amounts of oat malt in the backfill at different dosages of EP

Table 6. Physicochemical parameters of wort with varying amounts of oat malt in the mash (enzyme dosage: glucoamylase – 0.7 g/kg of malt, alpha-amylase – 1.0 g/kg of malt, combined enzyme preparation – 0.2 g/kg of malt).

Parameters	**Oat malt [%]**				
	90	**80**	**70**	**60**	**50**
Wort extract [%]	13,9±0,2	14,6±0,3	15,6±0,1	15,7±0,2	15,7±0,1
Wort volume [ml]	80±15	190±20	190±30	195±10	205±25
Density [g/cm3]	1,056 ±0,001	1,059 ±0,001	1,064 ±0,001	1,065 ±0,001	1,064 ±0,001
Viscosity [mPa*s]	1,75 ±0,01	1,78 ±0,02	1,80 ±0,02	1,83 ±0,01	1,79 ±0,01
Turbidity 90°[EBC]	55,6 ±2,3	28,3 ±1,7	13,1 ±3,3	10,6 ±2,3	6,0 ±1,1

Table 7. Physicochemical parameters of wort with varying amounts of oat malt (enzyme dosage: glucoamylase – 2.1 g/kg of malt, alpha-amylase – 2.4 g/kg of malt, combined enzyme preparation – 0.5 g/kg of malt).

Parameters	Oat malt [%]				
	90	**80**	**70**	**60**	**50**
Wort extract [%]	13,9±0,1	14,6±0,2	15,2±0,2	15,9±0,4	16,3±0,2
Wort volume[ml]	150±22	160±25	245±15	220±25	210±30
Density [g/cm3]	1,057 ±0,001	1,060 ±0,001	1,062 ±0,001	1,065 ±0,001	1,067 ±0,001
Viscosity [mPa*s]	1,703±0,02	1,698±0,01	1,723±0,01	1,756±0,01	1,809±0,02
Turbidity 90°[EBC]	103,3±5,4	47,7±4,4	13,7±3,2	8,3±2,1	5,2±0,7

As indicated in Tables 6 and 7, the best results were achieved using maximum dosages of fermented products (FP). When utilizing oat malt at proportions of 70%, 60%, and 50% in the oat-barley mash, the wort filtration rate increased by 1.5 to 1.6 times compared to the variants where the proportion of oat malt in the grist was 90% and 80%, and by 2.5 to 5 times higher than when mashing with 100% oat malt. In the combination of these malts at a 50/50 ratio, the filtration rate increased threefold compared to the result obtained when mashing with oat malt alone without the addition of barley malt; concurrently, the yield of high extract (HE) rose to 16.3%. Moreover, in the specified ratios (30/70, 40/60, 50/50), the turbidity level was reduced by 5 to 8 times. Saccharification was complete across all ratios.

5. Conclusion

In the conducted research work, the following results were achieved:

1) The use of 100% oat malt with varying ratios of exogenous enzyme preparations did not yield a satisfactory level of dry substance output, and the wort filtration rate was not optimal.

2) The study demonstrated that increasing the proportion of oat malt in the mixture with barley malt (beyond 50%) resulted in a decrease in the extractive value of the wort and an increase in turbidity. The optimal ratio for achieving the best filtration and extractive performance was found to be 65% oat malt and 35% barley malt.

3) The application of exogenous enzyme preparations (glucoamylase, alpha-amylase, and a combined preparation containing beta-glucanase and xylanase) improved the quality of the oat wort. However, satisfactory parameters for the oat wort were not achieved when using an economically viable dosage of enzyme preparations.

4) As evident from the experimental results, the highest extractive value (EV) of the wort, at 74.2%, was observed in the sample with the maximum enzyme dosage at a malt ratio of 50:50, which is characteristic of wort with these parameters. Based on these results, we recommend enzyme dosages of 2.5 g/kg of malt for alpha-amylase, 1 g/kg of malt for the combined preparation containing beta-glucanase and xylanase, and 2.5 g/kg of malt for glucoamylase.

5) A technology for producing wort from oat malt has been developed. This wort will subsequently be used as a material for the production of non-alcoholic and low-alcohol fermented beverages.

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