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Combining of Enzyme Preparations in Decoction Mashing and High Content of Maize Grits

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Running title: **Enzymes in Decoction Mashing with Maize Grits**

Abstract

The laboratory experiments have been carried out in order to obtain wort for special lager beer, comprising the use of maize grits up to 50%. A combination batch mashing method has been applied, including independent and joint use of enzyme preparations with combined and thermostable α-amylase activity. The benefit of adjusting the pH of the malt mash by improving a number of technological parameters has been proved. It is determined that there is a significant reduction in the dynamic viscosity of the wort and an increased utilization of the starch within combining enzyme preparations. A substantial increase in the content of α-amino nitrogen has been achieved while using a complex enzyme compared to the samples without such usage. In all the developed variants, it has been found that there is an undesirable reduction in the color of the wort within the gradual growth of the maize grits portion in the total malt grist.

Practical applications

It has been found that the application of thermostable α-amylase during the heat treatment of the surrogate batch and the addition of a complex enzyme preparation after mixing of the two mashes allows achieving very good technological characteristics with possibility for reduction of the beer cost but probably combined with a slight deterioration of its organoleptic characteristics..

Key words: maize, grits, malt, mashing, enzymes

Introduction

The application of various grain raw materials is widespread in the global brewing industry (Kunze, 2004). The use of unmalted raw materials has long been known and very common spread in practice. The use of surrogates leads primarily to significantly lowering of the cost of product (Briggs et al., 2004), and frequently to improving some technological and organoleptic parameters. However, same problems in the quality characteristics of the wort and beer can appear in cases of inappropriate combination of technological regimes and raw materials (Ignatov et al., 2015).

Maize grits is one of the most commonly used surrogates. It is characterized with high starch content and relatively few proteins, which makes it a desirable source of extract, especially with its lower price compared to that of the barley malt. Due to the high fat content in corn output (Kent, 1994), its processing into maize grits is mandatory and the fat content is limited to 1%. However, a higher temperature of the starch crystallization of over 70 ° C compared to that of the barley (Agu, 2002) has been observed. For better utilization of the maize grits the surrogate batch has to pass through braising (Eblinger, 2009) i.e. implementation of the decoction mashing methods. The lack of own active enzyme system in the maize grits requires application of a thermostable α-amylase during the heat treatment. For the same reason, a compensation of the strongly reduced malt enzyme systems may be needed depending on the ratio of barley malt and maize grits, using additional quantity of external enzyme preparations with a combined enzymatic activity that is often similar of that of the barley malt (Whitehurst et al., 2010).

The aim of the current study is tracking the changes in the technological parameters of the malt mash and the wort used for the production of light special beer. They have been received with an increasing share of the maize grits using independent or combined application of new enzyme preparations with diverse enzyme activity and combined decoction mashing method.

Materials and methods

Materials

Cereal raw materials

The experimental work has been performed using two types of grain raw materials - light barley malt produced in Austria and maize grits originating from Bulgaria. The data from the laboratory analysis are presented in **Table 1** and **2**.

Table 1. Characteristic of barley malt

Parameter	Unit	Result
Moisture	%	5,71
Saccharification time	min	15 ÷ 20
Hartong index 45°C		37,60
Extract content a.d.w.	%	83,22
Extract difference	%	1,43
pH		5,86
Color of wort	EBC	4,83
Viscosity of wort 8,6°P	mPa.s	1,48
Total protein	%	10,20
Diastatic power	W.K.	259,00
Kolbach index		41,00
α-amino nitrogen	mg/dm^3	201,27
α-amylase activity	U/g	175,00
β-glucans	mg/dm^3	187,00

Table 2. Characteristic of maize grits

Parameter	Unit	Result
Moisture	%	12,56
Extract content a.d.w.	%	91,26
Hartong index 45°C		36,85
Fats	%	0,91
Aflatoxin B1+B2+G1	µg/kg	0,19
Ochratoxin A	µg/kg	0,50
Fumonizin B1	µg/kg	50,00
Zearalenone ZEA	µg/kg	10,00
Zink	mg/kg	9,11
Lead	mg/kg	0,02
Cadmium	mg/kg	0,01
Screen №250 µm µm	%	4,74

Additional materials

During the course of mashing of the cereal mixture, the following two enzyme preparations have been applied - a thermostable α-amylase with temperature optimum of 85-90˚S and combined

enzyme preparation with a strong cytolytic and proteolytic activity.
The correction of the actual acidity is made using a lactic acid.

Staging of the experience

Few experimental variants with gradual increase of the content of the maize grits from 5% to 50% in the total malt grist have been developed. A combined decoction mashing method has been developed as a result of previously performed experiments and has been applied and presented in **Figure 1**. The regime is directly comply with the temperature optimum of the used enzyme preparations and the character of the grain raw materials.

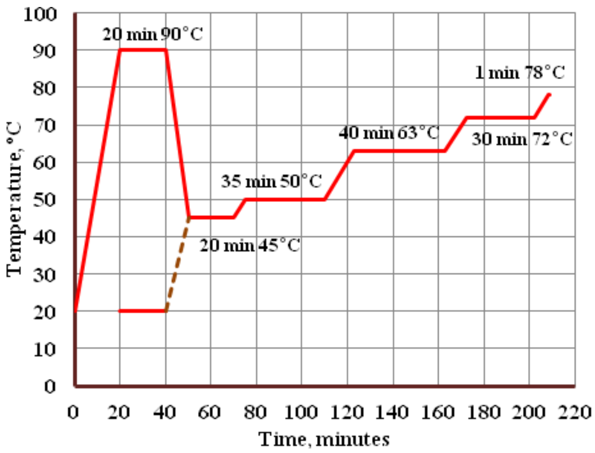

Figure 1. Combined decoction mashing method

The experiments have been conducted in two stages. The first stage includes mashing with the only application of a thermostable α-amylase during the boiling process of the surrogate batch. The second stage includes the use of combined enzyme preparation applied after mixing and cooling of the malt mash. The hydromodule between the used grain raw materials and water is complied with the desire to achieve a wort extract content at about 12%.
The studies have been conducted in laboratory conditions, by applying fine grinding of the malt with a disc grinder Buhler Miag DLFU.
Mashing has been done using a laboratory apparatus mash-Lochner LB-8 with a microprocessor controller, which allows a precise execution of the assigned mashing mode, as the boiling of the surrogate batch has been done separately.

Analytical methods

All the technological parameters of the resulting malt mash and wort have been carried out using generally accepted analytical methods of the European Brewery Convention (EBC-Analytica, 2005).

Results and discussion

In the initial stage of the conducted experiments using thermostable α-amylase, no adjustment of the pH of the malt has been realized. As a result, the actual acidity of the mash takes values which are far from optimal ones for all enzyme systems (pH 5,4 to 5,6). Therefore, some violations in the normal course of enzymatic hydrolysis processes can appear, and it is apparent from the data presented in **Figure 2**. Subsequently, an adjustment of the acidity using incorporation of lactic acid in all the experimental variants has been realized for improving the enzyme activity, which resulted in normalization of its values.

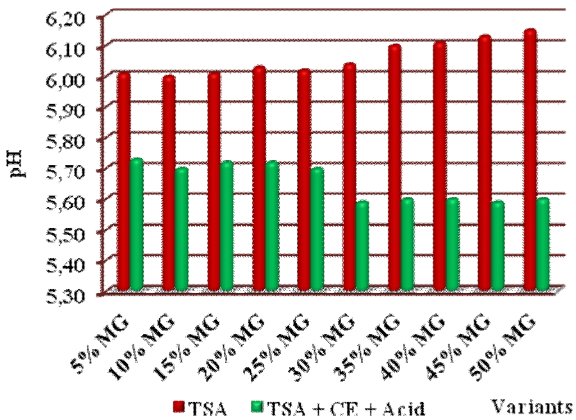

Figure 2. Changes of pH before and after addition of Lactic Acid

The improved conditions for the action of enzyme systems directly impacts the time for saccharification of the malt mash. The data presented in **Table 3** indicates that even without acidification, the use of thermostable α-amylase is enough for conductiong a normal saccharification of the mash, even with a significant increase in the maize grits in the mixture. However, as a result of the acidification, the time is significantly improved and adopt a stable value of 5-10 minutes in all variants.

Table 3. Saccharification time of mash

Variant	***Saccharification time, min***	
	With TSA	With TSA + CE + LA
5% MG : 95% BM	10 – 15	5 – 10
10% MG : 90% BM	10 – 15	5 – 10
15% MG : 85% BM	10 – 15	5 – 10
20% MG : 80% BM	10 – 15	5 – 10
25% MG : 75% BM	10 – 15	5 – 10
30% MG : 70% BM	10 – 15	5 – 10
35% MG : 65% BM	10 – 15	5 – 10
40% MG : 60% BM	10 – 15	5 – 10
45% MG : 55% BM	10 – 15	5 – 10
50% MG : 50% BM	10 – 15	5 – 10

The excellent saccharifying ability of the amylolytic enzyme complex of the malt, aided by thermostable α-amylase contributes significantly for achieving better extract content of the wort, even while using maize grits up to 50%, which is also main source of starch.

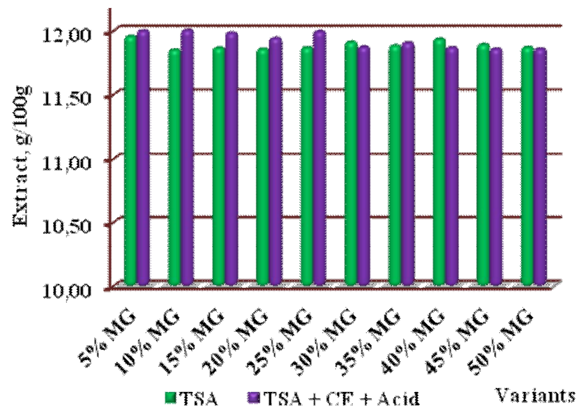

Figure 3. Changes of extract content of the wort

The results regarding the wort extract levels shown on **Figure 3**, clearly demonstrate the high efficiency of the enzyme preparations, including the cases when combined enzyme preparation featuring with lack of amylolytic activity have been used. Joint action of the two enzyme preparations increases the amount of low and middle molecular fractions of carbohydrates and nitrogenous substances, which actually form the bigger part of the wort extract. Probably, however, the minor differences in the extract are due to the redistribution and the different ratio of the products obtained by the enzyme hydrolize of different enzyme systems.

The gradual increase of the amount of maize grits in the common malt grist leads to smooth reduction of the dynamic viscosity of wort, since the lower values of the indicator are a prerequisite for accelerating the filtration of the malt mash.

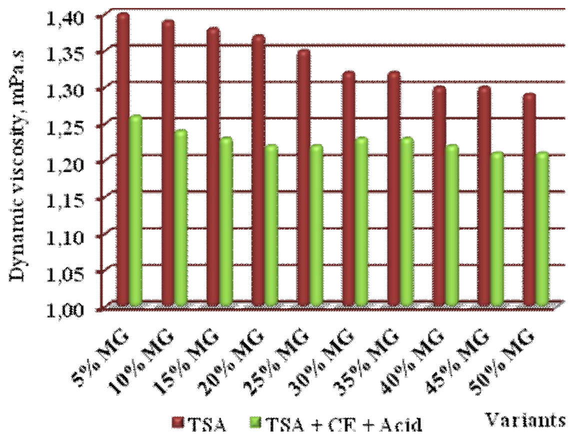

Figure 4. Changes of dynamic viscosity of the wort

The low viscosity is primarily result of the heat treatment of the surrogate batch, as well as the inclusion of termostable α-amylase. When combining the enzyme and temperature over 80 ° c it prevents the maize starch from claysterization, as well as from the potential possibility for increasing the viscosity.

The gradual reduction of the viscosity in all the variants is also due to the decreasing quality of the high molecular pentosans and hecsosans of the barley malt that pass in the wort extract.

The application of a combined enzyme preparation with underlined citolitic and proteolytic activity strongly shows its action by hydrolyzing the high molecular pentosanes and hecsosanes proteins. The reduction of their molecular mass leads to extremely serious reduction of the viscosity of the wort, and hence a significant acceleration of the filtration while using even 50% maize grits.

The results from the analyses regarding the content of α-amino acids in the wort, presented in **Figure 5** clearly show that the high pH of the wort, as well as the growing share of maize grits lead to deterioration of this indicator. It is apparent that in order to prevent potential problems in the fermentation process, the use of the grits should be

limited up to 30%. The addition of amylase does not affect the values of the indicator.
However, the content of α-amino nitrogen looks extremely different after acidification and addition of combined enzyme preparation.

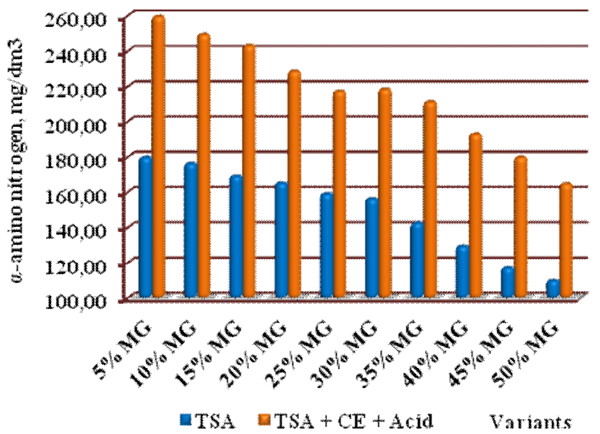

Figure 5. Changes of α-amino nitrogen of the wort

The decrease of the optimal pH values, as well as the proteolytic enzymes included in the complex contribute for the significant improvement of the indicator, which allows the use of maize grits to become possible within up to 50% in the total malt grist.
However, in terms of good visual organoleptic assessment of a light beer, the indicator wort color adopts relatively poor values.
The data in **Figure 6** demonstrates smoothly and surely decreasing in color, which is confirmed by our previous studies.

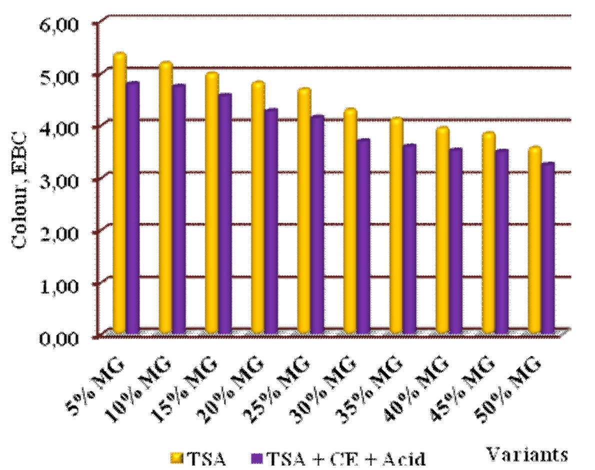

Figure 6. Changes of colour of the wort

The indicator undergoes a constant decrease within increaing the portion of the maize grits, no matter whether the optimum acidity has been reached or enzyme preparations with diverse activity have been incorporated.
This fact is largely due to the decreasing share of barley malt, which in practice is the main source of melanoidins and other aroma and taste compounds. In turn, this implies that in order to preserve the unique character of the color of light and special beers, it is better to limit the use of maize grits up to 30-35% in the common grist.

Conclusion
The current research applies a combined decoction method of mashing and usage of two types of enzyme preparations in gradual increase of the portion of the maize grits in the common malt grist. The purpose of the study is to establish the changes in the technological indicators of the wort aimed for production of special light beer under the conditions of the experiment.
As a result of the achieved tasks performed by the experimental set, the following main conclusions can be underlined:
1. A strict control of the pH of the mash and the wort as well as their timely correction are needed. The enhanced acidity significantly improves the saccharifying ability of the mash, even in cases of high quantities of surrogate.
2. The wort extract remains almost unaltered even after a significant increase in the participation of unmalted maize grits. The application of termostable α-amylase and heat treatment of the surrogate batch significantly improve the usability of the starch as a main source of extract.
3. When using a combined method of mashing, the viscosity of the wort adopts very good values, which have been improved significantly while using enzyme preparation with combined enzyme activity. The deep hydrolyse of the high molecular carbohydrates and proteins allows preserving the extremely good values of the indicator even when using 50% maize grits in the common mash.
4. It is not recommended to use more than 30% of the surrogate without using enzyme preparation with proteolytic activity. The concentration of α-amino acids has been significantly reduced while increasing the share of the surogate. The application of combined enzyme improves significantly the values of the indicator and allows the use of maize grits in higher doses.
5. Applying and increasing the amount of surogate does not affect positively the color of the wort, used for light special beer, even when combining enzyme preparations. From the point of view of

optimal color of the wort, it is recommended that a portion of maize grits in the common malt grist should be limited up to 30-35%.

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