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IMPACT OF POLYSACCHARIDES OF MALT ON FILTERABILITY OF BEER AND POSSIBILITIES FOR THEIR REDUCTION BY ENZYMATIC ADDITIVES

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ABSTRACT

Beta-glucans are principal constituents of barley endosperm cell walls. They are linear high-molecular polysaccharides, which tend to increase the solutions viscosity by forming gels. As β-glucans influence wort and beer viscosities, as well as beer quality, they are ones of the most studied wort and beer components.

This study is aimed at production of wort from malt at different degree of cytolytic modification and estimation of the effect of exogenous β-glucanases addition during wort fermentation. β-Glucans content in poorly- and well-modified malt was 1000 mg L^{-1} and 384 mg L^{-1}, respectively. The addition of enzymatic preparation with β-glucanases activity during the fermentation of wort, obtained from poorly modified malt, resulted in decreasing of beer viscosity by 5 – 40 % (depending on exogenous enzyme concentration: 1 and 2 BGU/L Finizym® 200 L) and decreasing of β-glucan content by more than 90 %.

Keywords: malt, wort, polysaccharides, β-glucans, exogenous β-glucanases.

INTRODUCTION

Polysaccharides are high-molecular carbohydrates consisted of monosaccharide residues linked by glucosidic bounds. According to their chemical composition and structure they are referred as: homopolysaccharides and heteropolysaccharaides [1, 2].

β-Glucans, being the main part of endosperm cell walls of barley grain - about 75 % of all carbohydrates, are ones of the most studied homopolysaccharides in brewing [3]. The inherent tendency of β-glucans to form gels cause increased wort and beer viscosity. At the same time the viscosities of wort and beer influence the brewing process and beer quality in opposite aspects [4]. At one side, beer viscosity positively contributes to its body and head retention of beer foams [5]. At the other side, high viscosities of wort and beer can lower the efficiency of many brewing operations and in large extent the process of beer filtration [6]. β-Glucans content in barley is reported to be between 0.4 and 8.6 % [6]. It depends on genotype of barley varieties, year, growing sites and environmental conditions [6-10].

Mixed linkage (1-3,1-4)-β-D-glucans, commonly known as β-glucans, are linear homopolymers of D-glucopyranosyl residues linked mostly via two or three consecutive β-(1-4) linkages that are separated by a single β-(1-3) linkage. Less frequent are longer segments of consecutively (1-4)-β-linked glucosil residues with degree of polymerization 5–28. There is no current evidence that two or more adjacent (1-3)-β-linkages occur in the β-glucan chains [11, 12]. 70 % of β-(1-4)- and 30 % of β-(1-3)- bounds are estimated in barley β-glucans (Fig. 1) [3,13-15]. Despite the non-random arrangement of individual (1-3) and (1-4)-β-linkages, the glucosil residues are arranged in an essentially independent and random fashion in the β-glucan chain [16,17].

Mixed-linkage β-glucans play mainly structural role in the barley grain. Cell walls of the starchy endosperm of barley consist of approximately 17 % of (1-3, 1-4) -β-D-glucans and 20 % of arabinoxylan, together with

smaller amounts of mannose-containing polysaccharides and cellulose [18]. Enzymatic hydrolysis (disintegration) of β-glucans is the most crucial brewing process. These polymers are broken down to various degrees mainly during malting [19]. The process is also known as malt modification. The cell walls are degraded by enzymatic complex referred as "citase". The complex comprises the enzymes: (1-3)-endo-β-glucanases; (1-4)-endo-β-gluconases; β-glucan-solubilases, and non-specific enzyme (1-3,1-4)-β-glucan endohydrolases also named (1-3,1-4)-β-glucanases; exo-β-glucanases and laminarinase [6,20]. As a result of cytolytic break down of cell walls during barley germination, other enzymes efficiently depolymerize the starch and other reserve proteins of endosperm. β-Gluconase is reported to be in very low quantity in barley grains, and their content dramatically increased after malting [10]. However, the endogenous barley β-glucanases synthesized during germination are damaged during mashing where temperatures around 50 °C are used. As a result β-glucans content in wort increased [5,21,22]. Thus, both the level of glucan-hydrolysing activities achieved during germination and the amount of β-glucans are important factors in the production of high quality malt [23]. The malt β-glucan content was reported to be more dependent on malt β-glucanase activity than on the original level of β-glucan in grains [10].

Incomplete disintegration of β-glucans has a negative impact on brewing. The presence of non-disintegrated high molecular β-glucans in wort increase viscosity of the wort and the resulting fermented beer, causing difficulties in filtration in the brewery. Reduced filterability of mash and beer has often been attributed to large β-glucans (molecular weight of 31 - 443 KDa) which tend to increase the viscosity of beer by forming inter-molecular hydrogen bonds between sequences of (1-4)-β-linkages [21,24-26]. Residual β-glucans may also play a role in beer maturation, promoting the formation of undesirable precipitate and hazes. β-Glucans content in barley is an indicator of malt modification and quality and illustrates effects of malting technology on quality of malt and wort [7,27]. As it was reported, wort viscosity varies from 1.59 to 5.16 mPa s and beer viscosity – between 1.45 and 1.96 mPa s [27].

Exogenous enzymes are used to supplement the malt's own enzymes in order to prevent filtration problems and β-glucan hazes [25, 28]. Addition of exogenous β-glucanases could be made during mashing-in or during wort fermentation [5, 23]. The commercial enzymatic preparations with β-glucanase activity are obtained by deep cultivation of special strains. Usually bacterial and fungal β-glucanases are used. The research on the application of commercial enzymatic preparations (exogenous enzymes) in brewing is focused mainly on the mashing and very scarcely on beer fermentation [5, 29-32].The reported technological effects of using commercial enzymatic preparations can be summarised as: reduced β-glucans content, better wort lauterring, increased extract yield and lowered beer haze.

This report presents the results from studying the effect of the addition of a commercial enzymatic preparation with β-glucanase activity during the fermentation of the wort, obtained from poorly modified malt.

EXPERIMENTAL

Materials

The malt used in this study is produced by two breweries A and B. The malts differed from each other in their degree of modification.

The commercial enzymatic preparation used was Finizym® 200L (Novo Nordisk, Denmark) – a fungal beta glucanase produced by a selected strain of Aspergillus niger. The enzymatic complex hydrolysis (disintegrated) barley β-glucans to oligosaccharides. The preparation is recommended for use during beer fermentation and

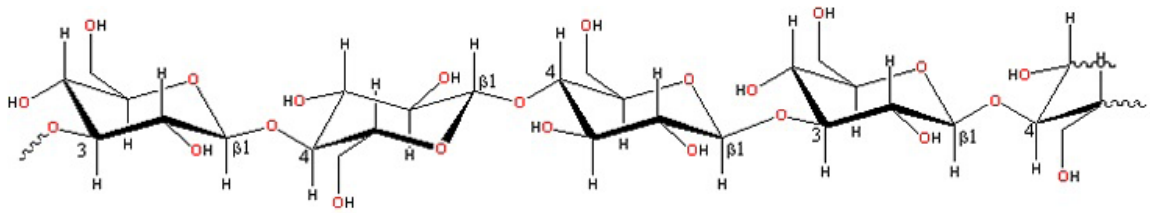

Fig. 1. β-Glucans structure.

maturing [33]. Tap water with residual alkalinity of 0.75 pH was used in brewing.

Methods

Ten wort samples were produced from different Bulgarian brewers. The worts were analysed and the viscosity and β-glucans content were determined.

Two malts obtained from the brewers A and B were used to obtain worts in lab scale experiments. The worts were fermented to obtain beer. The enzymatic preparation Finizym® 200 L was added only to worts, obtained from poorly modified malt.

Two lab-scale experiments were performed:

1) characterisation of beer obtained from malt at different extent of modification

The well modified malt supplied from brewery A was used as a control sample. The poorly modified malt supplied from brewery B was denoted as an experimental sample. 500 g of preliminary milled malt was mixed with water at 45°C and mashed following infusion method (Fig. 2).

At the end of the mashing, the weight of the mash was adjusted to 4500 g with distilled water and well homogenized. The obtained wort was separated by lautering. The wort was hopped with 80 mg L^{-1} α-bitter acids by boiling for 90 min. Yeast strain Saccharomyces carlsbergensis (4 g L^{-1}) was used in the fermentation at temperature 9-10 °C. At 10 % difference between "final apparent" and "apparent" degrees of fermentation, the obtained young beer was set to maturation at 4 °C for 20 days.

2) Study of the effect of enzymatic preparation Finizym® 200 L on brewing

Wort without enzymatic preparation was used as a control. The brewing process follows the same schema

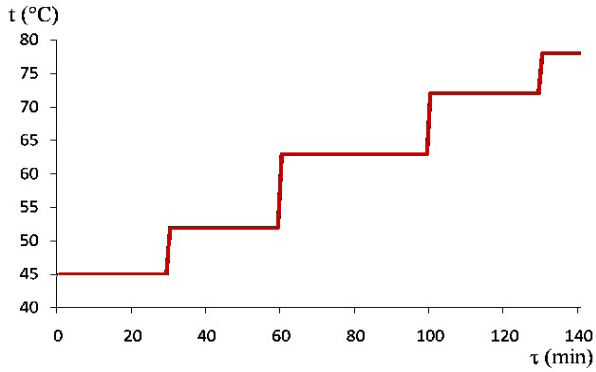

Fig. 2. Temperature program of infusion mashing.

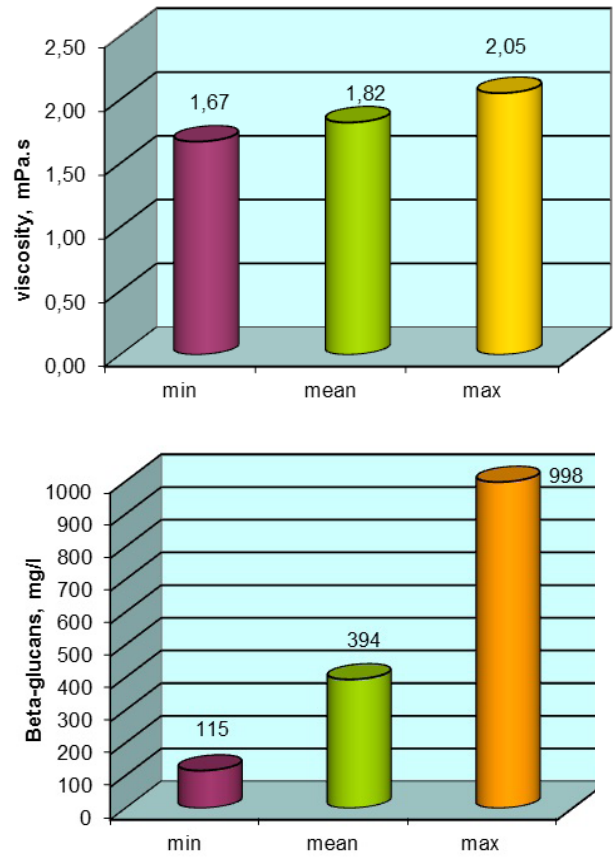

Fig. 3. Minimal, mean and maximal values of wort viscosity and wort β-glucans content of 10 wort samples, obtained from different breweries.

described above (section 1). The influence of quantity of added enzymatic preparation was studied at 1 and 2 BGU L^{-1} of enzyme. The Finizym® 200L was added to the wort in the start of fermentation.

Analysis

Standard analytical methods for beer quality assessment were used according to the European Brewing Convention [34]. β-Glucans were determined by spectrometric anthrone method for quantification of total carbohydrates [35].

RESULTS AND DISCUSSION

Estimation of the wort samples from different breweries in Bulgaria

Ten randomly chosen worts produced in Bulgaria were taken for the study of the range of wort β-glucan content and viscosity. The extract content in all studied

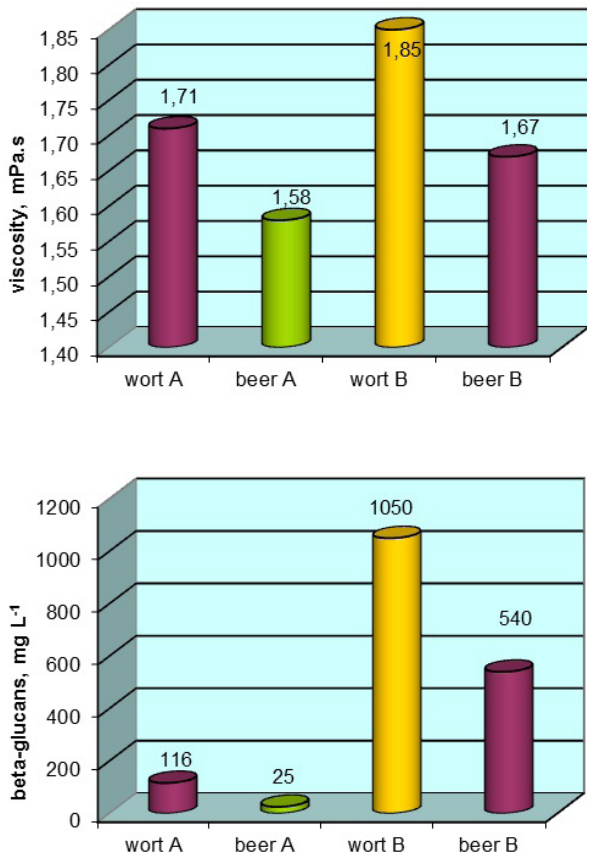

Fig. 4. Viscosity and β-glucan content in wort and beer obtained from: sample A – well modified malt; and sample B – poorly modified malt.

worts was 10.5 %. The results are presented in Fig. 3.

The wort viscosity ranged between 1.67 mPa s and 2.05 mPa s and the mean was 1.82 mPa s. The β-glucan content in wort was from 115 mg L^{-1} to 998 mg L^{-1} and mean was 394 mg L^{-1}. The β-glucans ratio calculated as a ratio of β-glucan content in well-modified malt to β-glucan content in poorly-modified malt was 1 to 2.7. Our results were lower from the reported in the literature ratio from 1 to 6 [36]. As it was mentioned in the introduction, quantity of released (solubilised) β-glucans during mashing depends on barley variety and malt modification during germination (β-glucan content and β-glucanases activity).

As the enzymes responsible for β-glucan hydrolysis were temperature depended [22], modifications of known mashing methods were not very efficient for lowering β-glucans content in wort. Malt quality, rather than the mashing method, was dominating factor determining the wort β-glucan level [5].

Evaluation of the malts samples

Quality of malts, produced by two breweries A and B, used for mashing in this study is presented in Table 1. As can be seen from the results, the malts differed from each other in their degree of modification. The viscosity, extract difference and β-glucan content of malt B were higher compared to the malt A. The value of viscosity of 1.63 mPa s (8.6 % Congress wort) was widely accepted as a reference for well-modified malt. Hence, the malt from brewery B was classified as "poorly modified" and the malts from brewery A - as "well modified".

Quality of beer obtained from poorly and well modified malt

The β-glucan content and viscosity of wort and beer, obtained from the above presented malts (A and B) are illustrated in Figure 4. As it was reported in the literature, an intensive cytolytic break down of β-glucans by the endogenous enzymes was observed in the temperature interval 35-51°C [5]. As can be seen form the results, β-glucans content in wort produced from poorly modified malt (B) was 1050 mg L^{-1} and decreased in beer down to 540 mg L^{-1}, whereas the β-glucans content in wort obtained from well-modified malt (A) and in produced beer was 116 mg L^{-1} and 25 mg L^{-1}, respectively. The β-glucans ratio in wort obtained from well- and poorly-modified malt was 1 to 9, higher than the β-glucan ratio in malts. The difference in β-glucan content in well- and poorly-modified malt couldn't be changed during mashing and the trend was the same. The viscosity of wort obtained from malt A and B was 1.71 and 1.85 mPa s, respectively, following the same trend as the β-glucans content: the higher viscosity was observed

Table 1. Quality of malts used for mashing.

	malt A	malt B
extract difference, %	1.0	2.9
viscosity, mPa s	1.58	1.70
β-glucan, mg L^{-1}	384	1040

in beer obtained from poorly modified malt (Fig. 4). The results coincided with the observation already made by other authors that the β-glucan content in wort depended mainly on the quality of malt [5]. For comparison we present practical criteria for good filterability of wort reported in the literature: viscosity below 1.65 mPa s and β-glucan content below 200 mg L^{-1} [7].

Quality of beer obtained with addition of exogenous enzymes

The addition of enzyme preparation Finizym® 200L at the beginning of the fermentation process was studied. The effect was estimated at two concentration levels of exogenous enzymes by comparing viscosity, β-glucan content and apparent degree of fermentation with control beer, produced without addition of enzymes. The quality of beer obtained with and without addition of enzymatic preparation Finizym® 200L is compared in Fig. 5. The obtained results showed that the addition of exogenous β-glucanases affected positively beer viscosity. The effect was more pronounced at 2 BGU L^{-1} of enzymatic preparation – around 40 %, compared to 5 % decreasing at 1 BGU L^{-1} level. Moreover, the addition of Finizym® 200L at concentrations 1 and 2 BGU L^{-1} increased apparent degree of fermentation up to 4.4 % and 5.2 %, respectively, compared to the control sample. The results indicate an improved fermentation of wort.

The most pronouncing effect of the addition of exogenous enzyme was observed on β-glucans content. It decreased by more than 90 % compared to the control beer. The results indicated a high degree of β-glucans break down. The highest activity of the exogenous enzyme Finizym® 200L was at the conditions of low temperatures and pH created during wort fermentation.

The obtained beer samples were also analysed to estimate the haze. Both samples B1 and B2 showed almost equals haze values, but 2 times lower than the control beer.

As the results showed, the beer filterability could be improved by addition of enzymatic preparation Finizym® 200L during fermentation of wort. Addition-

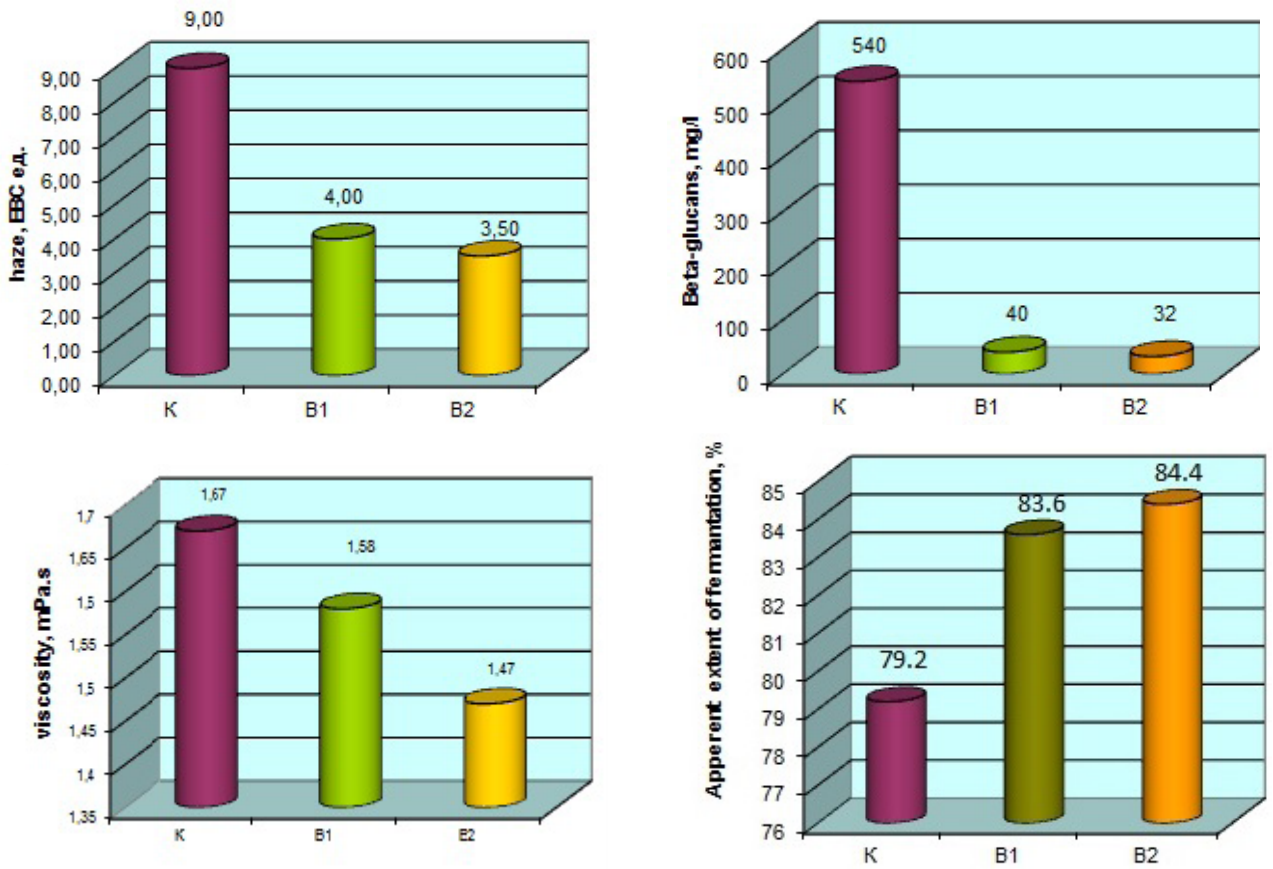

Fig. 5. Quality of beer obtained by addition of exogenous enzymes: K – control sample without exogenous enzimes; sample B1- with 1 BGU L^{-1} Finizym® 200 L; sample B2 - with 2 BGU L^{-1} Finizym® 200 L.

ally, the volume of the beer filtered by one pre-coat of kieselguhr filter can be increased.

CONCLUSIONS

The quality of ten batches of 10.5 %-wort produced by Bulgarian breweries was studied and the results showed that the wort viscosity and β-glucans content varied from 1.67 mPa s to 2.05 mPa s and from 115 to 998 mg L^{-1}, respectively. Two malts were produced in an industrial scale by two breweries. The cytolytic modification of the malt was characterized by viscosity, extract difference and β-glucan content. Based on their values the studied malts were classified as well- and poorly-modified. Both malts were used to obtain wort. A difference in the extent of β-glucans break down was observed. The β-glucan content in wort obtained from well- and poorly-modified malts was 116 and 1050 mg L^{-1}, respectively. The same trend was observed in the final beer. After addition of 1 or 2 BGU L^{-1} of enzymatic preparation Finizym® 200 L during wort fermentation, β-glucan content decreased by 90 %. The results showed that the addition of enzymatic preparation leads to high degree of enzymatic hydrolysis of biopolymers at the conditions of wort fermentation: low temperature and slightly acidic medium (pH around 5.0). Moreover, the addition of enzymatic preparation had a positive effect on brewing process lowering beer viscosity, improving beer fermentation and its colloidal stability.

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