# BIOCHEMICAL PROCESSES BY MASHING AND CHARACTERIZATION OF THE FERMENTATION OF FEED BARLEY DURING BREWING

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#### **ABSTRACT**

The purpose of the present work is to investigate the possibilities for the usage of feed barley in the brewing process. It is proved that using feed barley and the enzyme products Ceremix 2XL and Ultraflo L, the formed wort is richer in  $\alpha$ -amine nitrogen and the viscosity is almost the same as the one of the control liquid. No sufficient difference in fermentation ratio and period was found, comparing sample and control. The physical and chemical properties of the ready beer were almost the same as those of the control one. The most notable differences are concerning the vicinal diketones concentration.

Keywords: barley, wort, beer, enzymes, brewing yeast.

### INTRODUCTION

The quality of beer depends mostly on the composition and quality of the used raw materials. Barley is the basic brewing raw material. Its chemical composition, its brewing and technological indices are highly determinative for the beer quality and the economical efficiency of the brewing process. Due to the climate, only winter two-row barley brands are grown in Bulgaria. The lack of brewing barley can be compensated by using different four- or six-row feed barley forms. These types of plants can be used also for the purposes of malt production, which is actually the main byproduct in the brewing process. The barley can be classified as brewing or feed according to the structural properties of the plant [1]. The main quality index of the barley is its protein content. The feed barley is known for its high level of proteins, as well as high-molecular β-glucans [2]. The higher protein content leads to colloid instability of beer and lower extract yields. The quantity and molecular mass of the  $\beta$ -glucans influences the boiling yield, viscosity of the wort, and subsequently-its filtration [3-5].

When the malt shows enzymatic activity deficiency, different industrial enzyme mixtures are used. These products have cytolytic, proteolytic and amylolytic activities and are necessary for the high-molecular biopolymer degradation during the mashing. Thus, the composition of the wort is balanced.

The quality of the ready beer can be estimated by measuring the quantities of sugars, nitrogen containing compounds,  $\beta$ -glucans, etc. The main characteristics are the odor and taste potential. These characteristics depend on the substances that are formed as a result of the biochemical reactions during the fermentation of the wort. The process is catalyzed by the yeasts enzyme complexes.

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#### EXPERIMENTAL

Experiments were carried out on poly-row feed barley, Hemus brand, the control was – winter two-row brewing barley, Obzor brand. The Hemus brand was chosen following previous experiments. The protein content in the Obzor and Hemus brands was respectively – 12,0 % and 13,9 %. The water used in the experiment was taken directly from the city plumbing system. Its total hardness was  $5,04^{\circ}\text{H}$ , residual alkalis -  $0,66^{\circ}\text{H}$ , active acidity (pH) – 7,1.

The enzymes used for the present work were acquired from the Danish company Novozymes [6]. CERAMIX 2XL is a mixture of  $\alpha$ -amylase, neutral proteinase, and  $\beta$ -glucanase with respective enzyme efficiency - 488 KNU/g, 19 AU/g, 2616 BGU/g. ULTRAFLO L is a thermo-resistant multi-active  $\beta$ -glucanase product (423 BGU/g), that has also residual celulase, xylanase, pentosanase and arabinase activities. The dosage of the products was chosen from producer recommendations and our previous experience.

Experiments were carried out in semi-industrial conditions. The wort was obtained only from the malt and the mentioned enzyme products (K- Obzor brand,  $\rm B_1$ -Hemus brand,  $\rm B_2$ - Hemus brand and 0,04 g/100g CEREMIX 2XL and 0,10 g/100g ULTRAFLO L). The standard (infusion) mashing method was applied. A freshly prepared quantity of 500 g grist was used. The barley was grinded in a laboratory mill "Miag". The mixing with water was performed using hydromodule 1:3. The wort was then mixed with hoops, cooled and aerated.

The industrial yeast strain *Saccharomyces* carlsbergensis was used for the wort fermentation. The strain has a high fermenting and flocculating activity. The yeast cells were preliminary cultivated in standard wort. The inoculum contained 18-20 x 10<sup>6</sup> cells /ml. The temperature for the fermentation was around 9 – 10°C and the storage temperature 2 - 4°C for 20 days.

In all the experiments standard methods for the Republic of Bulgaria and the European Brewing Convention (EBC) were used.

#### RESULTS AND DISSCUSION

The feed brand has lower qualitative indices than the Obzor brand. Table 1 shows the physical and chemical properties of the resulting malts. The extractable content is lower by 3 %, and the protein content is higher by 1,8 %. This is a prerequisite for the higher quantity of water soluble nitrogen 105 mg/100g. The extracting difference shows that there is insufficient seed modification, especially its cytolytic degradation. This can be concluded also after examination of the viscosity. The same saccharification time for the sample and the control brand depicts a similar amylolytic degradation.

Table 2 shows the physicochemical composition of the wort. The control showed the longest saccharification period (Obzor brand). The higher value of this index discovered with experimenting on  $\mathbf{B}_1$  is most probably due to higher amylolytic potential. The addition of the enzyme products (type 2), additionally lowers this value [2]. The turbidity of the wort is de-

|                                       | Brand of Barley |       |  |
|---------------------------------------|-----------------|-------|--|
| Index                                 | Obzor           | Hemus |  |
| Moisture, %                           | 4,9             | 4,2   |  |
| Extract of fine grist, %              | 80,7            | 77,5  |  |
| Extract difference, %                 | 2,0             | 3,2   |  |
| Proteins, %                           | 11,3            | 13,1  |  |
| Saccharification time, min            | 10-15           | 10-15 |  |
| Viscosity, mPa.s (8,6% Congress wort) | 1,57            | 1,82  |  |
| Color, (EBC)                          | 2,5             | 2,5   |  |
| Water soluble nitrogen, mg/100g       | 595             | 700   |  |

| Index                      | <sup>1)</sup> K | $^{2)}$ B <sub>1</sub> | $^{3)}$ B <sub>2</sub> |
|----------------------------|-----------------|------------------------|------------------------|
| Saccharification time, min | 10-15           | 5-10                   | 5                      |
| Extract content,%          | 10,36           | 10,50                  | 10,79                  |
| Turbidity, (EBC)           | 2,30            | 2,20                   | 1,90                   |
| pН                         | 5,60            | 5,60                   | 5,65                   |
| Color, (EBC)               | 7,5             | 8,5                    | 6,5                    |
| Fermentation ratio, %      | 78,4            | 79,0                   | 80,5                   |
| Viscosity, mPa.s           | 1,64            | 1,80                   | 1,61                   |

Table 2. Physical and chemical composition of the wort.



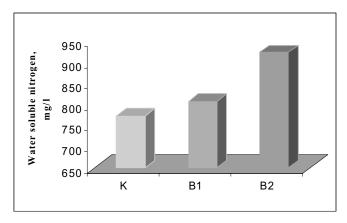


Fig. 1. Water soluble nitrogen.

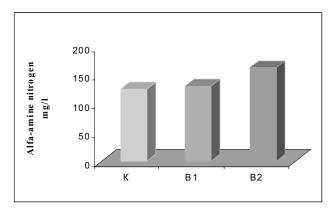


Fig. 2. Alfa-amine nitrogen.

creasing in both of the types and has its lowest value for type 2.

Another important index is the clarification degree. The turbidity (presence of solid particles) indicates a high lipid, and especially - fatty acids content. The latter are vital yeast cell membrane components and

can be synthesized aerobically only [7]. Despite the different level of cytolytic degradation, the acquired data shows that the use of the enzyme complexes results in a normal viscosity value in type 2. The water soluble nitrogen value has its peak when using wort type 2. The same relation is noticed when looking at the quantity of  $\alpha$ -

| Parameters                                                                                                           | <sup>1)</sup> K | <sup>2)</sup> B <sub>1</sub> | $^{3)}$ B <sub>2</sub> |  |
|----------------------------------------------------------------------------------------------------------------------|-----------------|------------------------------|------------------------|--|
| Apparent level of fermentation, %                                                                                    | 78,05           | 78,85                        | 79,75                  |  |
| Difference between final and apparent level of fermentation, %                                                       | 0,25            | 0,15                         | 0,60                   |  |
| <sup>1)</sup> K- Control Obzor, <sup>2)</sup> B <sub>1</sub> – Hemus, <sup>3)</sup> B <sub>2</sub> – Hemus + enzymes |                 |                              |                        |  |

Table 3. Characterization of yeast fermentation activity.

Table 4. Physical and chemical composition of the beer.

| Parameters                                                                                                           | <sup>1)</sup> K | <sup>2)</sup> B <sub>1</sub> | $^{3)}B_{2}$ |  |
|----------------------------------------------------------------------------------------------------------------------|-----------------|------------------------------|--------------|--|
| Original extract, %                                                                                                  | 10,55           | 10,65                        | 10,40        |  |
| Apparent extract, %                                                                                                  | 2,35            | 2,25                         | 2,10         |  |
| Intrinsic extract, %                                                                                                 | 3,79            | 3,51                         | 3,55         |  |
| Alcohol, %                                                                                                           | 3,44            | 3,52                         | 3,50         |  |
| Apparent degree of fermentation, %                                                                                   | 78,20           | 78,90                        | 79,80        |  |
| Color,                                                                                                               | 7,0             | 8,0                          | 8,0          |  |
| Viscosity, mPa.s                                                                                                     | 1,60            | 1,70                         | 1,58         |  |
| β-glucans, mg/l                                                                                                      | 101             | 121                          | 80           |  |
| Water soluble nitrogen, mg/l                                                                                         | 480             | 580                          | 630          |  |
| α-amine nitrogen, mg/l                                                                                               | 46              | 58                           | 70           |  |
| Vicinal diketones, mg/l                                                                                              | 0,31            | 0,23                         | 0,14         |  |
| Esters, mg/l                                                                                                         | 20              | 22                           | 16           |  |
| Fusel alcohols, mg/l                                                                                                 | 54              | 60                           | 65           |  |
| <sup>1)</sup> K- Control Obzor, <sup>2)</sup> B <sub>1</sub> – Hemus, <sup>3)</sup> B <sub>2</sub> – Hemus + enzymes |                 |                              |              |  |

amine nitrogen (Fig. 1 and Fig. 2). Amino acids responsible for odor and taste metabolites formation are of highest importance. The influence of the amino acid profile and concentration is important for the various biochemical reactions in which they take part [8]. The results for the concentrations of Valin, Isoleucine and Leucin, showed slight differences between the control and the sample wort. Therefore, we could state that the usage of feed barley instead of brewing one leads to a change in the quantity of free oxygen and has a negligible influence over the amino acid profile.

The cell concentration data is shown on Figs. 3 and 4. The X axis shows the period of fermentation and the Y axis - the wort extract deviation. The figures for the specific growth rate ( $\mu$ ) are as follows: K - 0,0326,

 $B_1$  - 0,0290,  $B_2$  - 0,0298. This shows that  $\mu$  value of the control is higher than in  $B_1$  and  $B_2$  (respectively the yeast generation time in the control is significantly shorter). The cell concentrations measured on the  $48^{th}$  hour are respectively 61, 54, 50 cells x  $10^6/ml$ . The maximum concentration was observed between the  $2^{nd}$  and the  $4^{th}$  day. It can be seen on Figs. 3 and 4 that by the  $96^{th}$  hour of the process the fermentation activity of the yeast is high. The percentages of the used fermentable extract for K,  $B_1$ ,  $B_2$  are respectively 69 %, 70 %, 66 %. This can also be seen in Table 3 – apparent level of fermentation of the fresh beer (168 hours). All these results show that there is no significant difference between the fermentation rate of the control and the sample wort. The yeast retain their high fermentation activity not only

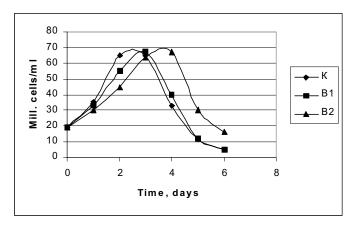


Fig. 3. Changes in cell concentration during the fermentation of the wort

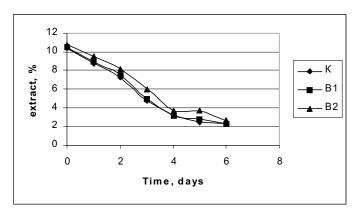


Fig. 4. Changes in the extract content during the fermentation of the wort.

during the active phase, but also during the second half of the process. There are some slight differences though.

The physical and chemical composition of the ready beer is listed in Table 4. The different beers show almost no difference concerning the apparent and the intrinsic alcohol and extract. The viscosity keeps up the trend established during the wort experiments. Type 2 has a viscosity value similar to the control. The same pattern is followed by the  $\beta$ -glucans. As the  $\alpha$ -amine nitrogen is assimilated during the fermentation process, its quantity is lower compared to the wort. The degree of assimilation of the  $\alpha$ -amine nitrogen is K- 63,5 %, B<sub>1</sub> – 55.7 %, B<sub>2</sub> – 57,3 %. The overall results reveal that the fermentation of the control and sample wort influence the yeast biosynthetic activity significantly. This is true especially for the vicinal diketones. The quantity of fusel alcohols and esters are in the expected range.

## **CONCLUSIONS**

In our experiment we used feed barley and the enzyme products Ceremix 2XL and Ultraflo L. We compared the produced wort to a control one and established that it had higher quantity of soluble and  $\alpha$ -amine nitrogen, and similar viscosity. The rate and degree of fermentation of the control and sample wort did not differ significantly. The physical and chemical parameters of the beer were almost the same as those of the control. The concentration of the vicinal diketones showed the greatest difference in the compared control and beer samples.

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