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 α-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 vinyl acetate diketones concentration.

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

INTRODUCTION

The quality of the beer depends mostly on the composition and the 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 for- 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 lead to colloid instability of beer and lower extract yields. The quantity and molecular weight of the β-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, 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, β-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.
The purpose of the present work was to investigate the possibilities for a usage of feed barley in brewing processes.

MATERIALS AND METHODS

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 according to previous experiments. The protein content in 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 ^{o}H, residual alkalis 0.66 ^{o}H, pH = 7.1.
The enzymes used for the present work were acquired from the Danish company Novozymes [6]. CERAMIX 2XL is a mixture of α-amylase, neutral proteinase, and β-glucanase with respective enzyme efficiency: 488 KNU/g, 19 AU/g, 2616 BGU/g. ULTRAFLO L is a thermo-resistant multi-active β-glucanase product (423 BGU/g), that has also residual cellulase, xylanase, pentosanase and arabinase activities. The dosage of the products was evaluated by the firm recommendations and our previous experience [7].
Experiments were carried out in semi-industrial conditions. The wort was obtained only from the malt and the mentioned enzyme products (K- Obzor brand, B_1- Hemus brand,

B_2- Hemus brand and 0.04 g/100 g CEREMIX 2XL and 0.10 g/ 100g ULTRAFLO L). Standard (infusion) mashing method was applied. 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.

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 $(18 – 20).10^6$ cells/mL. The temperature for the fermentation was around 9 – 10 ^oC and the storage temperature 2 – 4 ^oC for 20 days.

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

RESULTS AND DISSCUSIONS

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/100 g. The extracting difference shows that there is insufficient seed modification, especially its cytolytic degradation. This also can be concluded after examination of the viscosity. The same saccharification time for the sample and control brand depicts a similar amylolytic degradation.

Table 2 shows the physicochemical composition of the wort.

Table 1. *Technological Characteristic of the Malt*

Index	Brand of Barley	
	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 /100 g	595	700

Table 2. *Physical and Chemical Composition of the Wort*

Index	[1)]К	[2)]B_1	[3)]B_2
Saccharification time, min	10-15	5-10	5
Extract content, %	10,36	10,50	10,79
Turbidity, (EBC)	2,30	2,20	1,90
pH	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

[1)]**К**- Control Obzor, [2)]B_1 – Hemus, [3)]B_2 – Hemus + enzymes

The control showed the longest saccharification period (Obzor brand). The higher value of this index discovered with experimenting on 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 decreasing 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 high lipid, and especially fatty acids content. The latter are vital yeast cell membrane components and can be synthesized aerobically only [8]. Despite the different level of cytolytic degradation, the acquired data shows that the use of the enzyme complexes results in 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 discussing the quantity of α-amine nitrogen (fig. 1 and fig. 2). Amino acids responsible for odor and taste metabolites formation are of most importance. The influence of amino acid profile and concentration is important for the various biochemical reactions in which they take part [8].

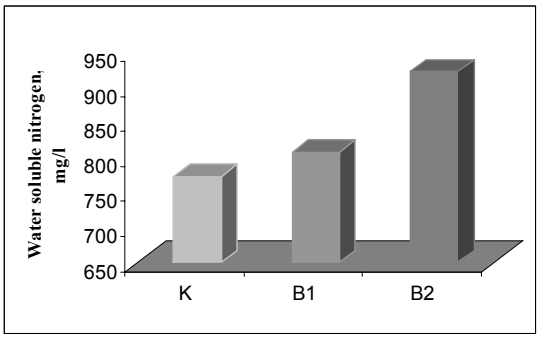

Figure 1. *Water soluble nitrogen*

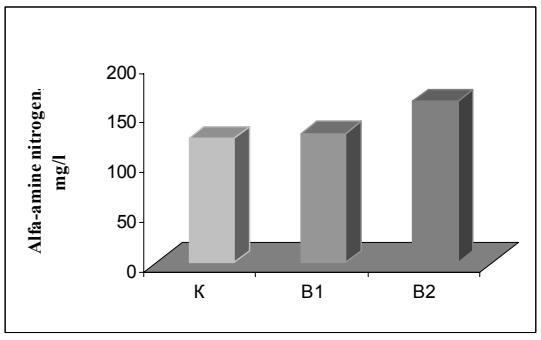

Figure 2. *Alfa-amine nitrogen*

The results for the concentrations of valine, isoleucine and leucine, 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 are shown in figures 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 (μ) are as follows: K – 0.0326, B_1 – 0.0290, B_2 – 0.0298. This shows that μ 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 in the 48th hour are respectively 61.10^6, 54.10^6, 50.10^6 cells/mL. The maximum concentration was observed between the 2nd and the 4th day. It can be seen on figures 3 and 4 that by the 96th hour of the process the fermentation activity of the yeast is high. The percentage 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 this results show that there is no significant difference between the fermentation rate of the control and the sample wort.

Table 3. *Characterization of Yeast Fermentation Activity*

Parameters	K	B_1	B_2
Apparent level of fermentation, %	78.05	78.85	79.90
Difference between final and apparent level of fermentation, %	0.25	0.15	0.60

[1] **K**- Control Obzor, [2]**B_1** – Hemus, [3]**B_2** – Hemus + enzymes

The yeast retain their high fermentation activity not only 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 viscosity value similar to the control. The same regularity is followed by the β-glucans. As the α-amine nitrogen is assimilated during the fermentation process, its quantity is lower compared to the wort. The degree of assimilation of the α-amine nitrogen is K – 63.5%, B_1 – 55.7%, B_2 – 57.3%.

Table 4. *Physical and Chemical Composition of the Beer*

Parameters	K	B_1	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, %	78.2	78.9	79.8
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

[1] **K**- Control Obzor, [2]**B_1** – Hemus, [3]**B_2** – Hemus + enzymes

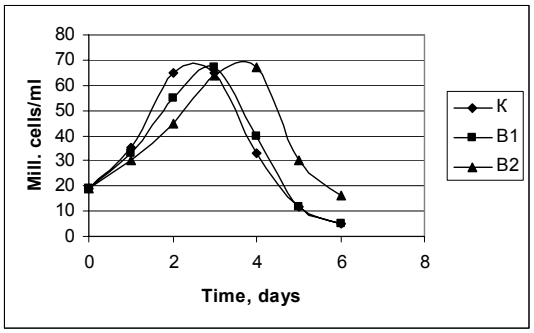

Figure 3. *Changes in cell concentration during the fermentation of the wort*

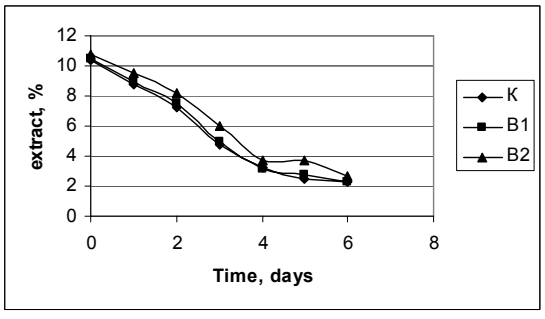

Figure 4. *Changes in the extract content during the fermentation of the wort*

The overall results reveal that the fermentation of the control and sample wort influences 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 has higher quantity of soluble and α-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 sample.

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