DYNAMICS OF CHANGES IN THE PROTEIN PROFILE OF BARLEY GRAIN DURING THE BREWING PROCESS USING DIFFERENTIATED FERTILIZATION WITH NITROGEN AND SULPHUR

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ABSTRACT

Our objective was to check out the effect of nitrogen fertilizers and nitrogen fertilizers with sulphur in nutrition of spring barley (variety Bojos). The quality of protein complex was determined on grain samples. The application of sulphur showed also in the fractional composition of the proteinous complex. The representation of high-molecular D-hordeins (S-rich) and the LMW glutelins (S-rich) was demonstrably increased in variants with applied sulphur. The sum of albumin, globulin fraction and D-hordeins in wholemeal groats moved between 13.4 – 17.7 %. The changes of fractional protein composition caused by degradation of high-molecular compounds during malting were confirmed. The content of D-hordeins, soluble albumins and globulins in the malt in comparison with barley corn is markedly increased (increase between 7.3 – 14.1 %). There is a hypothesis for increase of contents of soluble nitrogen in the wort, which is indispensable for propagation of yeast and fast fermentation.

Key words: barley, protein, HPLC, malt, sweet wort

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INTRODUCTION

Sulphur belongs to significant essential nutrients and as such it is indispensable for the growth and development of plants. It is an important consideration concerning vegetal metabolism, its lack has a negative effect on the quality of harvest (Ceccotti et al., 1997; Zhao et al., 1999). The necessity of sulphur for barley is by 80-90 % used for the creation of sulphurous amino acids. The compounds containing sulphur are an important factors when aroma and taste of beer is formed, especially when producing a lager type (Anness, Bamforth, 1982; Kuktaite, 2004). The aim of this article is to demonstrate whether a differential nutriment by nitrogen and sulphur can influence the content of proteinous fractions in a barley grain and the dynamics of their changes during the malting and mashing process.

MATERIAL AND METHODS

The experiment is carried out on a piece of land belonging to the collective farm Agrospol Velká Bystřice cadaster. The spring barley Bojos was used after the sugar beet for the experiment. The sowing was made on March 23, 2012 and it amounted to 4 MGS. The application of nitrogen fertilizers and nitrogen fertilizers with the sulphur was carried out in accordance with the scheme stated in the chart No.1. Each of the variants was repeated four times, the gross size of plots was 21.6 m^2 and it was modified for the harvest to 14.3 m^2 (13 x 1.1 m).

Tab. 1 Experiment variants

Term application	DC 13		DC 31		In sum (kg.ha^{-1})	
Variant	Applied fertilizer	N (kg.ha^{-1})	Applied fertilizer	N (kg.ha^{-1})	N	S
1	-	0	0		0	0
2	LAV 27	30			30	0
3	LAV 27	30	DAM	20	50	0
4	SA	30			30	36
5	SA	30	SAM	20	50	42
6	DASA	30			30	15
7	DASA	30	SAM	20	50	21
8	SAM	30			30	10
9	SAM	30	SAM	20	50	16
10	LAV + S1	30			30	30
11	LAV + S1	30	DAM	20	50	30
12	LAV + S2	30			30	50
13	LAV + S2	30	DAM	20	50	50

Comment: LAV 27 – Ammonium nitrate with limestone (27 % N, 20 % CaO, SA – ammonium sulphate (20.3 % N, 24 % S), DASA (26 % N, 13 % S), SAM (19 % N, 6 % S), DAM (30 % N), S1, S2 – elemental sulphur (1, 2 – dose).

The barley crop was harvested in full maturity with the help of a thrasher Wintersteiger for small plots. The analysis of a proportional content of the single proteinous fraction (glutenins, hordeins, albumins, globulins) in the proteinous complex of a barley grain, malt and sweet wort was carried out with the help of AgilentChemstation for LC and LC/MS Systems software. Evaluation was carried out taking into account the work of Celus et al. (2006).

RESULT AND DISCUSSION

The content of the single protein groups depends on the total content of proteins in the barley. Hulín et al. (2008) presents: albumins 12.1 %, globulins 8.4 %, prolamins 25 % and glutenins 54.5 %. The content of high-molecular D-hordeins rich in sulphur provably increased concerning the variants where sulphur was applied. The amount of the albumin and globulin fractions and D-hordeins in wholegrain meal oscillated in the range of 13.4 – 17.7 % (fig. 1).

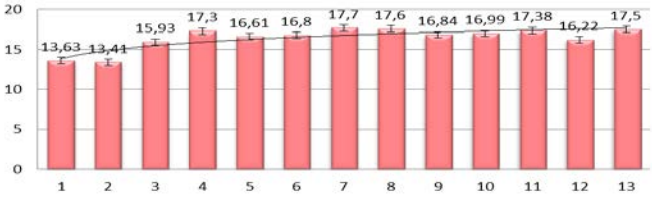

Fig. 1: D-hordeins, albumins a globulins in barley grain (%)

The prolamins were divided, depending on their structural and functional qualities, into three groups namely S-Poor (sulphur-deficient: D-hordeins), S-Rich (rich in sulphur: B and γ hordeins) and HMW (high molecular weight: D-hordeins). Only the B and C hordein groups can be considered typical hordeins. They differ not only by their formula weight, but have different content of sulphurous amino acids of cystein (Černý, Šašek, 1998).

The content of a sulphur-deficient sub fraction of C-hordeins was relatively balanced and oscillated in a range of 8.2 – 8.9 %. B-hordeins are rich in sulphur prolamins, their molecular weight ranges 32 – 45 kDa and they are the biggest hordein fraction (80 %). Against the assumption the effect of the sulphur applied was not vindicated, the highest content could be seen at variants 1-3 without the applied sulphur (picture 7).

Glutelins were the least explored proteinaceous fraction of the grain, what is caused by their poor dissolubility and so highly effective dissolving agents and extractive conditions are necessary, what can often cause their denaturation, possibly degradation (Wilson et al., 1981).

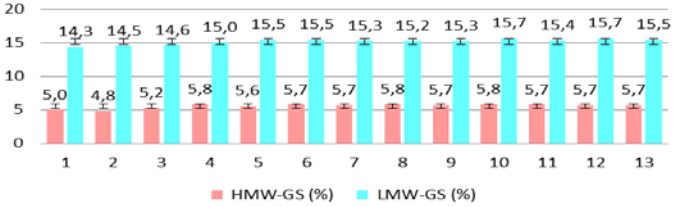

Fig. 2: Low- and high- molecular weight glutelins in barley grain (%)

The content of LMW subunits of glutelins, that are designated as S-rich, showed growing trend depending on the dose of the sulphur applied. Their content was the highest with variant 10 and 12

with the lower level of fertilization by nitrogen and the sulphur applied in the dose of 30 and 50 kg.ha^{-1}. The content of HMW glutelin subunits was relatively balanced in the range 4.8 – 5.7 %, and again a slightly growing trend can be seen depending on the application of sulphur (fig. 2).

Considering that dimethylsuphid and S-methyl-L-methion contain the sulphur, we can assume, that their concentration is affected by the content of sulphur in caryopsis. The study Zhao et al. (1996) implies that malty quality of the grain is significantly affected by application of the sulphur and there occurs the increase of hydrolytic enzymes activity. And simultaneously the concentration of DMS precursors on malt increases. But if the dose of the sulphur is adequate and meets the requirements of the plants, the concentration does not increase and the assumptions for higher content of PDMS in the malt are not created (Hřivna et al., 2010).

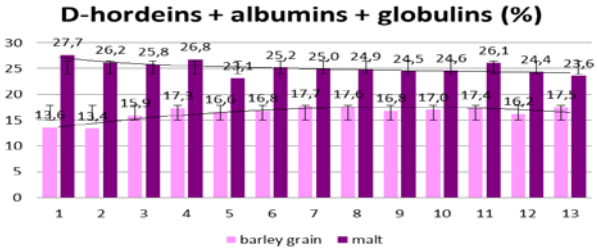

Fig. 3: D-hordeins, albumins a globulins in barley grain and malt (%)

As it is evident from the figure 3, the content of D – hordeins, albumins and globulins in the malt is markedly increasing in comparison with the barley grain, the content of albumins and other soluble proteins is increasing during germination through degradation of high molecular proteins. This forms the hypothesis of increasing content of soluble nitrogen in the malt. From the stated fractions is the substantial importance attached to the ß – globulin which takes part due to the low value of the isoelectric point 4.9 which is near to pH of beer and the high content of cysteine in formation of un-biological turbidity in the beer (Görg et al., 1992; Basařová et al., 2010). The representation of low-molecular and high-molecular glutelin subunits has not considerably changed in comparison with the barleycorn (picture 2), due to their above mentioned attributes (fig. 4).

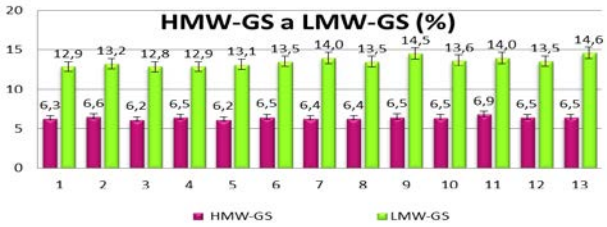

Fig. 4: Low- and high- molecular weight glutelins in malt (%)

The representation of the C – hordeins which are poor in sulphur was balanced in all variants and it was ranging between 9.1 – 9.6 %. The content of B – hordeins (S:rich) in the malt is substantially noticeably reduced, it is coming to their degradation into the low-molecular compounds. Their representation in particular variants is equal (fig. 5).

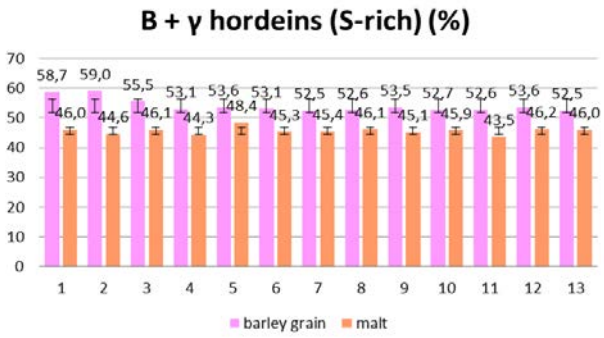

Fig. 5: B-hordeins (%)

Only about 20 % of the total protein content in the corn is water soluble and resistant in relation with the protelyosis and thermic coagulation and thanks to this it goes to beer unchanged (Osman et al., 2003). In order to gain a brewing of a good quality, it is necessary to change a part of insoluble proteins to soluble ones during the time of storing and mashing. This fraction consists of amino acids, peptides and soluble proteins; a big part of them is created just with the help of barley protein proteolysis (Jones, Budde, 2005). Thanks to the fact they contain of D-hordeins, albumins and globulins in the sweet wort (in comparison with malt) noticeably increased, what is positive. Their content was not noticeably different in comparison with the single variants and it was in range of 31.0 – 33.3 % (fig. 6).

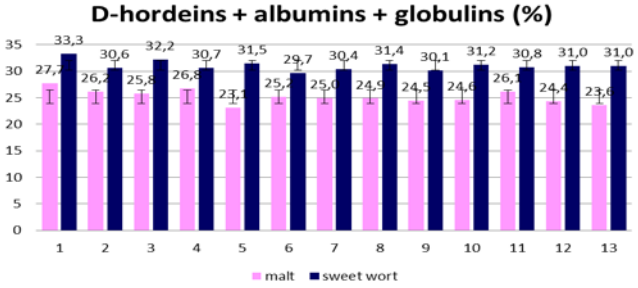

Fig. 6: The representation of water soluble proteins in malt and sweet wort (%)

Glutelins form at about 30% of barley proteins. They can be found solely in a corn endosperm. They are not ruptured by mashing and they go the malt unchanged, what confirms the results of the study of Briggs, Hough, 1981. During the brewing process the B-hordeins (S-rich) degradation goes on and their content in the wash is still reduced and their degradation to lower molecular compounds comes up. Their representation at the single variants is in range 37.2 – 40.0 % (fig. 7). The changes of another protein fractions presented in malt and sweet wort were not significant.

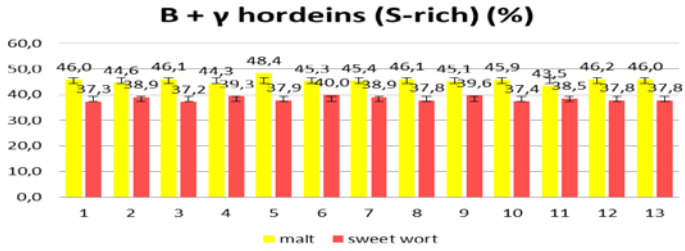

Fig. 7: The representation of B-hordeins in malt and sweet wort (%)

CONCLUSIONS

Within the experiment the proteinaceous profile of barley and its changes during the brewing process was analysed. The content of high-molecular D-hordeins (S-rich) and low-molecular subunits of glutelins in a barley grain has provably increased when dealing with the variant where sulphur was applied. The sum of albumin and globulin fractions and D-hordeins in wholewheat groats was in range 13.4 – 17.7 %. The changes of fractional protein composition caused by high-molecular compounds degradation during storing were confirmed. The content of D-hordeins, albumins, globulins solvable in malt is significantly increasing in comparison with a barley grain (the growth in range 7.3 – 14.1 %). The degradation of high-molecular compounds penetrates through the whole brewing process and thanks to this the content of D-hordeins, albumins, globulins in wash (in comparison with malt) further provably increased, what is positive. So there is assumption for increase of the content of nitrogen solvable in wort and it is necessary for the process of yeast breeding and for fast fermentation.

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