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## SURVEY OF $\beta$ -GLUCANS IN DOMESTIC BARLEY'S VARIETIES

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### ABSTRACT

$\beta$ -glucans in cereals are desirable, but malting and brewing industries appreciate lower levels of these compounds because high level of  $\beta$ -glucans in barley varieties can cause unsatisfactory degradation of cell walls during malting. Low to moderate  $\beta$ -glucan content in barley is preferable for malt production. The aim of this study was to investigate the share of  $\beta$ -glucans in 16 Croatian barley varieties at three representative locations in eastern Croatia: Osijek, Slavonski Brod and Tovarnik over three consecutive seasons (2012-2014). Total  $\beta$ -glucan content in barley samples was determined using enzymatic method. Overall, total  $\beta$ -glucan contents ranged between 2.21 and 4.50 g/100 g dry weight, where barley feed variety had the highest and malting barley variety had the lowest content. Most of the investigated barley varieties had total  $\beta$ -glucan content lower or significantly lower than 4 %. Barley varieties used for livestock (feed and hulless varieties) appear to have the highest (feed > 4.4, hulless 4.6 > g/100 g), and brewing varieties have the lowest  $\beta$ -glucan (< 3.6 g/100 g).

**Keywords:**  $\beta$ -glucans; barley, brewing quality

### INTRODUCTION

$\beta$ -glucans appear to beneficial effects on human health and that is why many research have been opened regarding this subject. However, from brewers' perspective,  $\beta$ -glucans are not an appealing component in cereals intended for malting and brewing.

$\beta$ -glucans are non-starch polysaccharides characterized by (1 $\rightarrow$ 3), (1 $\rightarrow$ 4)  $\beta$ -D-glucose linkage (Vis and Lorenz, 1997) and are main constituents of endosperm cell walls. They can be found in barley and oats, while in other cereals (wheat) they are present to a significantly lesser extent (Vis and Lorenz, 1997). Total content of  $\beta$ -glucans in barley normally ranges from 2 to 8 % (Marconi *et al.*, 2014) and depends on genetic factors, but climatic conditions, agrotechnical measures and soil type also contribute to the total  $\beta$ -glucan content in barley (Zheng *et al.*, 2000; Aastrup, 1979; Narziss *et al.*, 1999).  $\beta$ -glucans, in small amounts, contribute to beer foam stability and improve beer organoleptic properties (i.e. beer mouth feel) (Havlová *et al.*, 2006). However, in higher levels they cause serious problems during both, malting and brewing. During malting,

high total  $\beta$ -glucan content can lead to unsatisfactory degradation of cell walls, which disrupts the germination and reduces the malt extract (Wang *et al.*, 2004).  $\beta$ -glucans residues in malt can lead to poor mash conversion, resulting in highly viscous wort. This can cause problems during the filtration process (Vis and Lorenz, 1998; Wang *et al.*, 2004) and induce haze in beer (Jin, 2002).

This is why barley with low to moderate  $\beta$ -glucan content is preferable for malt production (Vis and Lorenz, 1998). It is interesting that the existing research results suggest the use of six-rowed barley that has somewhat lower  $\beta$ -glucan content than the two-rowed varieties (Lehtonen and Aikasalo, 1987; Zhang *et al.*, 2001). Based on the intended end use in respect to the characteristics of barley varieties, they can be classified as malting, feed and malting-feed. Multipurpose varieties are interesting to the producers because most of the varieties are winter varieties with somewhat higher yields (30% higher) and lower cost compared to spring varieties. However, in order to be acceptable for malting/brewing, main quality parameters have to be suitable, such as  $\beta$ -glucan content, protein share, friability, glassy grains share.

The objective of this study was to determine the total  $\beta$ -glucan content in 16 barley varieties collected at three locations over three consecutive seasons (2012-2014). The acquired data will then serve maltsters as an important input information upon the admission of barley for malting.

## MATERIALS AND METHODS

*Samples.* Samples of 16 different barley varieties (Rex (P/S), Barun (P/S), Bingo (S), Bravo (S), Maxim (P/S), Premium (P/S), Gazda (P/S), Lukas (P/S), Maestro (P/S), Merkur (P/S), Trenk (P/S), Lord (P/S), Tiffany (P), Vanessa (P), Matko (GZ), GZ-184 (GZ)) were collected over three consecutive seasons (2012–2014) from variety trials (Agricultural Institute, Osijek) at three representative locations in eastern Croatia: Osijek (OS), Slavonski Brod (SB), and Tovarnik (TO). Labels P/S for brewing and feed varieties, S for feed varieties and B for brewing varieties describe the purpose for which a certain variety can be classified. Sampling (5 kg per sample) was performed on cleaned and processed barley grains (according to EBC 3.1. method) and the samples were kept refrigerated in sterile dry containers. Soil types at locations were: eutric cambisol (OS), alluvial soil (OS) and hipogley soil (TO). All varieties are winter two-rowed varieties that originate from Agricultural Institute Osijek, except for varieties Tiffany and Vanessa that originate from Germany and variety Lord that is six-rowed.

*Determination of total  $\beta$ -glucan content.* Prior to  $\beta$ -glucan determination the samples were milled using standard laboratory knife mill with 1 mm sieve (MF10.2 basic, IKA Labortechnik, Germany) and after that using kitchen coffee grinder (Braun KMM 10). Barley flour samples were kept in sealed plastic bags until  $\beta$ -glucan content determination. Total  $\beta$ -glucan content in barley was determined according to enzymatic method (AOAC Method 995.16) using a commercial assay kit (Mixed Linkage determination kit, Megazyme International Ireland, Bray, Ireland).

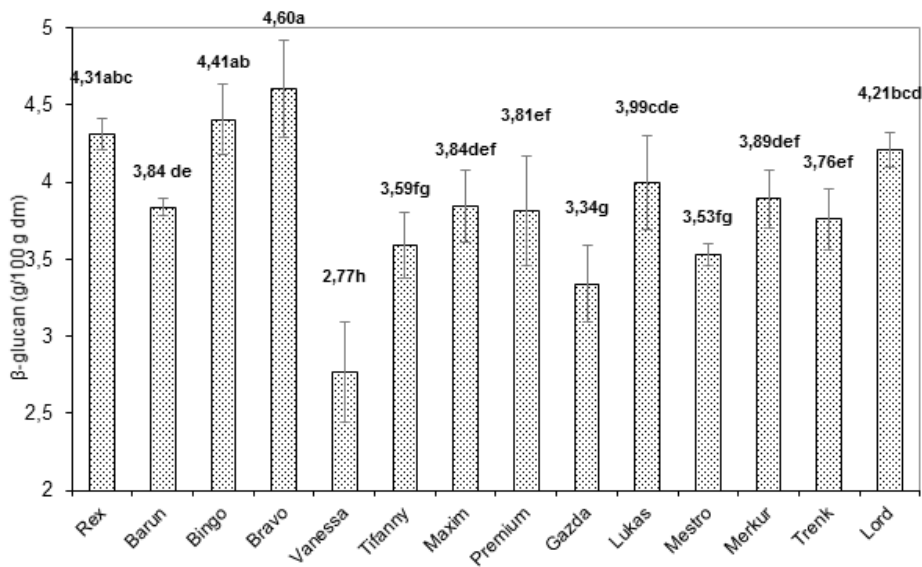
*Statistical analysis.* Statistical analysis was carried out using Statistica Ver. 8.0 StatSoft Inc. Tulsa, OK, USA. The impact of individual factors (variety, location, intended use) on differences in average values of  $\beta$ -glucan were analysed using the analysis of variance (ANOVA) and the Fisher's least significant difference test (LSD), with statistical significance being set at  $P < 0.05$ .

## RESULTS AND DISCUSSION

Croatian varieties of different traits and grain properties were investigated in this research. Most biotic and abiotic stress in Croatian varieties is caused by diseases, acidic soil, cold weather, draught, frequent extreme temperatures, and rainfall during germination and growth period. High temperatures during May and June are also known to affect the intensity and grain filling period and can cause forced maturation of the grain (Lalić *et al.*, 2006; Lalić *et al.*, 2007), the most unfavourable abiotic stress. Forced maturation can lead to the increase in total  $\beta$ -glucan content of barley (Passarella *et al.*, 2002). Both genotype and environmental conditions have an impact on the content of  $\beta$ -glucans, although genetic factors seem to be of more importance (Molina-Cano *et al.*, 1997).

Malting procedure is time limited, meaning that  $\beta$ -glucan degradation takes place at germination temperatures, the only period in which enzymes degrade  $\beta$ -glucans. Solubility of  $\beta$ -glucans is greatly affected by the structure and interrelations of certain  $\beta$ -glucan fractions. Starting concentration of  $\beta$ -glucan content in grains correlates with  $\beta$ -glucan concentration in wort, but applied brewing technological procedure contributes to final  $\beta$ -glucan content in wort. Even though there are no recommendations for brewers regarding the total  $\beta$ -glucan content in malt, when it comes to wort it is recommended it should not exceed 200 mg/L (Bamfort, 2006).

American malting Barley Association has more stringent recommendations. Program for barley development sets recommended  $\beta$ -glucan concentrations in wort at  $< 100$  mg/L for two-row barley and  $< 120$  mg/L for six-row barley (AMBA, 2014). However, in practice greater values are tolerated (Malt specifications & brewing performance: IGB (Institute & Guild of Brewing (methods)  $< 200$  mg/L, or EBC (European Brewery Convention)  $< 250$  mg/L). Nevertheless, it is often hard to achieve the recommended values because the total  $\beta$ -glucan content of the starting raw material (barley) is about 4 % (EBC, 1998; MEBAK, 1997). For three consecutive years (2012-2014) total  $\beta$ -glucan content was analyzed in chosen barley varieties, and the results are given in Fig. 1. The results show that total  $\beta$ -glucan content of the majority of varieties was lower or significantly lower than 4 %. Five varieties, namely Bingo, Bravo, Rex, Lord and Lukas had total  $\beta$ -glucan content higher than 4 %. This can be explained by the fact that latter are early-maturing varieties and thus avoid the forced maturation caused by draught period.



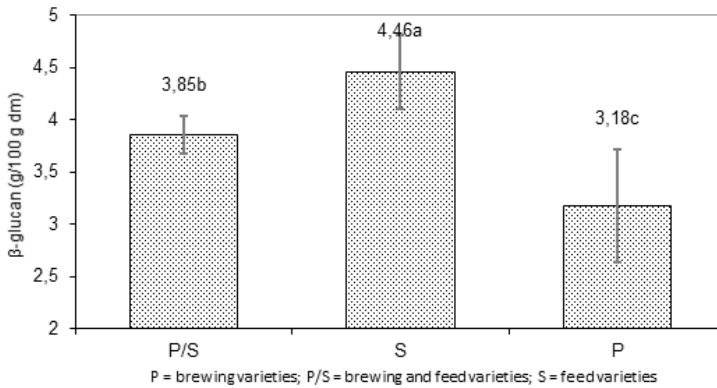
**Figure 1.** Three-year (2012-2014) average total  $\beta$ -glucan content in analysed barley varieties.

Climatic conditions typical for Croatia and South-eastern Europe are shown in **Tab. 1**. Genotypes with later heading date and longer vegetation period (Tiffany, Lord and Vanessa) mostly have lower yields in regard to earlier heading date varieties (Bingo, Barun and Maxim) (Lalić *et al.*, 2003; Lalić *et al.*, 2006). Lowest total  $\beta$ -glucan content (<3 %) was determined for variety Vanessa at all three investigated locations. This was expected, since this German variety is classified as strictly malting variety. Variety Tiffany, also a German malting variety, had significantly higher total  $\beta$ -glucan content than Vanessa, but still less than 4 %. Even though not classified as strictly malting varieties but as a combined malting-feed varieties, Gazda and Maestro also had higher total  $\beta$ -glucan in regards to malting varieties. Maestro gave narrow and Gazda wider dispersion of the total  $\beta$ -glucan content over the entire investigated period. A clear differentiation of varieties on the basis of their intended purpose (i.e. malting, feed and malting-feed) was noticed considering the  $\beta$ -glucan share. This was expected since the earlier investigation of the same varieties showed significant differences between other quality parameters (total and soluble nitrogen, albumin and globulin fractions of proteins, extract, 1000 kernels weight etc.) (Kovačević *et al.*, 2008). When varieties were grouped according to their end use (Fig. 2), a distinct and clear genotype influence can be noticed regarding the total  $\beta$ -glucan content. Varieties inside one group differ statistically, which reflects to differences between groups.

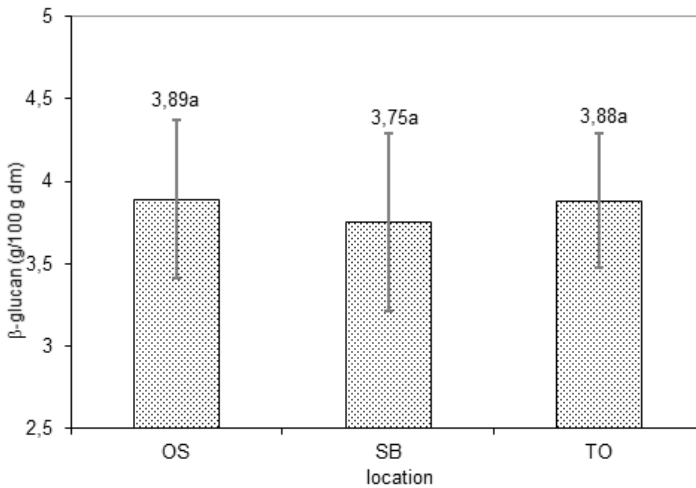
**Table 1.** Climatic data on mean monthly air temperatures and rainfall for investigated period 2011/12 – 2013/14 for Osijek, Slavonski Brod and Tovarnik locations

location	<b>OSIJEK (TO)</b>								
	temperature of air (°C)				rainfall (mm)				
year	<b>2011</b>	<b>2012</b>	<b>2013</b>	<b>2014</b>	<b>2011</b>	<b>2012</b>	<b>2013</b>	<b>2014</b>	
mean	11.7	12.3	12.1	12.8	-	-	-	-	
total	-	-	-	-	422.2	599.2	767.2	809.4	
vegetation period X - VI	8.3		9.1	10.2		392.3		679.0	573.2
location	<b>SL. BROD (SB)</b>								
	temperature of air (°C)				rainfall (mm)				
year	<b>2011</b>	<b>2012</b>	<b>2013</b>	<b>2014</b>	<b>2011</b>	<b>2012</b>	<b>2013</b>	<b>2014</b>	
mean	11.6	12.4	11.9	12.6	-	-	-	-	
total	-	-	-	-	432.9	640	738.8	962.9	
vegetation period X - VI	8.3		9.1	9.9		419.5		647.2	567.9
location	<b>TOVARNIK (TO)</b>								
	temperature of air (°C)				rainfall (mm)				
year	<b>2011</b>	<b>2012</b>	<b>2013</b>	<b>2014</b>	<b>2011</b>	<b>2012</b>	<b>2013</b>	<b>2014</b>	
mean	12.1	12.9	12.6	13.4	-	-	-	-	
total	-	-	-	-	397.4	448.4	733.4	824.0	
vegetation period X - VI	8.9		9.6	10.8		332.4		625.3	460.9

(source: Državni hidrometeorološki zavod Hrvatske / Meteorological and Hydrological Service of State)



**Figure 2.** Three-year (2012-2014) average total  $\beta$ -glucan content in analysed barley varieties in respect to intended use classification.



**Figure 3.** Three-year (2012-2014) average total  $\beta$ -glucan content in analysed barley varieties. in respect to growth location.

Out of many environmental factors, this investigation followed only the influence of location on total  $\beta$ -glucan share. No statistically significant influence of location on the total  $\beta$ -glucan share has been determined during this three-year experiment (Fig. 3).

However, the differences between the varieties at each location were noticed depending on the year, confirming the impact of genotype on the total  $\beta$ -glucan share. All the above listed varieties were grown at trial fields, subjected to identical agro-technical measures and weather conditions. Varieties grouping around the three-year average of total  $\beta$ -glucan content at all three locations is given in **Fig.3**. A clear distinction between varieties (based on their end use) can be seen. Variety Gazda, as previously stated, has singled out from the combined malting-feed group and joined the malting group with Vanessa and Tiffany. Feed group varieties have also singled out, while the majority of varieties in the malting-feed group clustered around the mean value. Apart from Vanessa and Tiffany, specifically designed German malting varieties, the results showed that a large number of the investigated Croatian malting-feed varieties can also be acceptable for brewing in respect to their total  $\beta$ -glucan content. Two investigated varieties of hulles barley were excluded from data processing. Namely, even though they gave acceptable results for  $\beta$ -glucan content in starting barley,  $\beta$ -glucan content is usually unacceptably high in wort.  $\beta$ -glucan content in Matko was 4.62 g/100g dm and in GZ-184 4.05 g/100g dm. Variety GZ-184 is borderline acceptable according to  $\beta$ -glucan content in grains, but after malting  $\beta$ -glucan content amounted to high 320 mg/L while the amount of  $\beta$ -glucan in Matko was totally unacceptable with 500 mg/L.

## CONCLUSIONS

Results of this study indicate that barley varieties classified as feed varieties (used as livestock feed) contain highest total  $\beta$ -glucan content ( $> 4.4$  g  $\beta$ -glucan /100 g dry weight), and malting varieties had the lowest total  $\beta$ -glucan content ( $< 3.6$  g  $\beta$ -glucan /100 g dry weight). Total  $\beta$ -glucan content of seven out of ten varieties classified as malting-feed varieties ranged from 3.75 – 4.25 g  $\beta$ -glucan /100 g dry weight. Gazda and Maestro, two malting-feed varieties had total  $\beta$ -glucan content significantly lower than 4 %, which makes them suitable for malting. Significant differentiation of varieties was determined based on their total  $\beta$ -glucan content, while location had no statistically significant impact.

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