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# β-GLUCAN CONTENT AND β-GLUCANASE ACTIVITY OF WINTER AND SPRING MALTING BARLEY CULTIVARS

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β-Glucan content and β-glucanase activity of winter and spring barley cultivars grown under different environments were evaluated. There were significant differences in both β-glucan content and β-glucanase activity between analysed barleys. The results showed that, for all cultivars and locations, approximately 75% of β-glucan present in grains was degraded after malting, and that marked differences existed among winter and spring type of cultivars in malt β-glucan content. The correlation analysis of β-glucan content and malt quality parameters showed that malt β-glucan content was significantly positively correlated with viscosity and extract difference, and negatively with malt β-glucanase activity and friability. Regarding malt β-glucanase activity, significantly higher activity was found in spring cultivars in contrast to winter cultivars.

**Keywords:** barley, β-glucan content, β-glucanase activity, malting quality, winter and spring cultivars

Barley is the primary cereal used in the production of malt in the world. During malting, barley undergoes an incomplete natural germination process that involves a series of enzyme degradations of the barley kernel material, of which the degradation of cell wall components belongs to the most important ones (Gamlath et al., 2008). Cereal β-glucans are linear homopolysaccharides made of glucose with approximately 70% (1→4)-linkages and 30% (1→3)-linkages, located mainly in the cell walls of the endosperm and the aleurone layer (Holtekjølen et al., 2006).

The aim of malsters is to produce malt by modifying barley endosperm as efficiently as possible. As a component of the walls of endosperm cells, in high concentrations, β-glucan may lead to insufficient degradation of cell walls during malting, which in turn obstructs the diffusion of germination enzymes and the mobilization of kernel reserves, and hence reduces malt extract content (Zhang et al., 2001; Wang et al., 2004). The degradation of endosperm cell walls and subsequent changes in β-glucan levels during malting are, to a great extent, related to β-glucanase activity, which depolymerises β-glucan. The changes of both β-glucan content and β-glucanase activity in barley during malting are interesting to breeders and malt producers, as both of them are closely associated with malt yield and quality. Thus, during malting, β-glucan content shows substantial decline, accompanied by an increase in β-glucanase activity. Therefore, better malting performance is expected to be associated with lower levels of β-glucan in grains and higher levels of β-glucanase in malt (Wang et al., 2004).

There have been several studies on the genetic and environmental variation of both β-glucan content and β-glucanase activity. Lehtonen and Aikasalo (1987) reported that two-row barley genotypes had higher β-glucan content than six-row barleys, and showed clear differences existed between barleys grown at different locations in Finland. Narasimhalu

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and co-workers (1995) found significantly higher β-glucan contents in barleys from eastern compared with western Canada. Perez-Vendrell and co-workers (1996) showed that significant differences existed in β-glucan content of 10 two- and six-row barley cultivars between genotypes, locations, and years. Stuart and co-workers (1988) showed that the β-glucan content was greatly depolymerised by β-glucanase during malting. Thus, the low β-glucan content at the end of malting was the most important indicator of malting quality rather than the original content in grain. Wang and co-workers (2004) found out that after malting β-glucanase activity was dramatically increased in malt when compared to low levels of β-glucanase activity detected in barley grains, and that there were significant differences among both cultivars and locations. The objective of this work was to select different seasonal type barley cultivars and to study the content of β-glucan in barley seed and in malted barley grains. Statistical analysis was used to determine the differences in β-glucan content and β-glucanase activity between winter and spring barley cultivars grown at different locations.

## 1. Materials and methods

Samples of three winter cultivars (Zlatko, Barun, and Vanessa) and three spring cultivars (Fran, Matej, and Scarlett) were collected from experimental fields of the Agricultural Institute Osijek at two locations, Osijek and Nova Gradiška in year 2009. Cultivars Zlatko, Barun, Fran, and Matej were created at Agricultural Institute Osijek in Croatia. Winter cultivar Vanessa and spring cultivar Scarlett were recognized at Josef Breun GDBR – Herzogenrauch breeding station in Germany.

Prior to micromalting, the barley samples were screened over a 2.5 mm sieve and analysed for crude protein content by non-destructive near infrared transmission method (Infratec 1241 Grain Analyzer). Malt analyses were done according to the European brewery convention (EBC) official methods (Analytica-EBC, 1998). The following parameters were determined: malt extract content, extract difference between fine and coarse ground malt, Kolbach index, diastatic power, viscosity, and friability. The mixed linked β-glucan contents in barley grain and malt samples and β-glucanase activity in finished malt were determined using Megazyme assay kits (Megazyme Ltd., Ireland). Results are averages of duplicate measurements. Statistical analysis of data was done with statistical-graphic system *Statistica 8.0* (Stat Soft Inc. software, USA).

## 2. Results and discussion

The mean values of barley grain and malt quality parameters are presented in Tables 1 and 2. Low protein and β-glucan contents are desirable qualities in malting barley. Barley suitable for malting should have grain protein content lower than 11.5%, as high protein content will not only reduce malt extract, but also deteriorate final beer quality (Qi et al., 2005). The results of this study showed that protein content was quite low when compared with normal commercial requirements, being on average lower than 11% for all cultivars and locations. Thus, protein content should not be a factor affecting malting quality of barley samples included in this study. Spring cultivars had malt extract content higher than winter cultivars, what is in accordance with our previous report (Šimić et al., 2007). In malting, the activity of the enzymes, which degrade grain starch, is determined in terms of diastatic power, being a

measure of the malt capacity to hydrolyse starch into fermentable sugars (Zhang et al., 2006). In our study, winter barley cultivars had a significantly higher diastatic power than spring barley cultivars.

The polymeric β-glucans are broken down to various degrees during malting and mashing. When high molecular weight β-glucans are solubilised from the cell walls, these polymers increase the viscosity of wort and the resulting fermented beer (Jin et al., 2004; López-Perea et al., 2012). Results showed significant differences between winter and spring cultivars in the extent of cytolytic modification of endosperm during malting. Cytolysis describes the degradation of the supporting and structural cell wall substances in the coating of the starch-bearing cells of the endosperm. Differences were evident from mean values of viscosity, extract difference between fine and coarse ground malt, and friability. Although some variation was present in the values for Kolbach index, these did not differ significantly between the winter and spring cultivars and the locations.

*Table 1.* Mean values for barley grain protein, malt extract, and extract difference between fine and coarse ground malt, Kolbach index, and diastatic power

| Cultivar/ Location/ Seasonal type | Grain protein (%) | Malt extract (%) | Fine/Coarse difference (%) | Kolbach index (%) | Diastatic power (U.W.-K.) |
|---|---|---|---|---|---|
| Zlatko | 11.13 $^{a*}$ | 80.60 $^{a}$ | 2.85 $^{a}$ | 38.60 $^{a}$ | 287.5 $^{b}$ |
| Barun | 10.64 $^{a}$ | 80.80 $^{a}$ | 2.45 $^{ab}$ | 39.65 $^{a}$ | 297.5 $^{b}$ |
| Vanessa | 10.77 $^{a}$ | 82.20 $^{a}$ | 1.35 $^{bc}$ | 40.00 $^{a}$ | 364.5 $^{a}$ |
| Fran | 10.95 $^{a}$ | 81.55 $^{a}$ | 1.10 $^{c}$ | 37.85 $^{a}$ | 158.0 c |
| Matej | 10.89 $^{a}$ | 81.35 $^{a}$ | 1.15 $^{c}$ | 37.65 $^{a}$ | 191.0 $^{c}$ |
| Scarlett | 10.96 $^{a}$ | 82.05 $^{a}$ | 0.85 $^{c}$ | 43.15 $^{a}$ | 316.0 $^{ab}$ |
| Osijek | 10.99 $^{a}$ | 82.02 $^{a}$ | 1.57 $^{a}$ | 39.07 $^{a}$ | 281.7 $^{a}$ |
| Nova Gradiška | 10.79 $^{a}$ | 80.83 $^{a}$ | 1.68 $^{a}$ | 39.90 $^{a}$ | 256.5 $^{a}$ |
| Winter | 10.84 $^{a}$ | 81.20 $^{a}$ | 2.22 $^{a}$ | 39.42 $^{a}$ | 316.5 $^{a}$ |
| Spring | 10.93 $^{a}$ | 81.65 $^{a}$ | 1.03 $^{b}$ | 39.55 $^{a}$ | 221.7 $^{b}$ |
| Average | 10.89 | 81.43 | 1.63 | 39.48 | 269.1 |

$^{*}$: significant difference ($P \leq 0.05$) is indicated by different letters

β-Glucan contents in barley grains and malt are shown in Table 2. After malting, the β-glucan content in malt was dramatically reduced in comparison to content in grains. The total mean β-glucan content in malt, across locations and cultivars, was 1.29%, being 25.7% of the total content in grains. This means that approximately 75% of β-glucan content present in grains was degraded during malting. However, there was a marked difference among cultivars for the degraded portion of β-glucan content during malting. For instance, only 10.6% of grain β-glucan content remained in malt after malting for cultivar Scarlett, averaged over locations, correspondingly it was 34.7% and 38.8% for cultivars Vanessa and Barun, respectively. In addition, it can be seen from Table 2 that the difference among winter and spring cultivars in β-glucan content was significantly larger in malt than that in grains, and

β-glucan content in malt was to a great extent dependent on its degradation during malting rather than the original level in grains. For instance, cultivar Scarlett had the lowest content of β-glucan remained in malt, even though it had the highest grain β-glucan content. Change of β-glucan content during malting has been reported. ELLIS and co-workers (1997) found that the breakdown of β-glucan content in grain happened with the development of β-glucanase activity, and the total grain β-glucan content declined by about 50% after malting. STUART and co-workers (1988) showed that the β-glucan content was greatly depolymerised by β-glucanase during malting. Thus, the low β-glucan content at the end of malting was the most important indicator of malting quality rather than the original content in grain. WANG and co-workers (2004) showed in their study that, on average for all cultivars and locations, approximately 80% of β-glucan content present in grains was degraded during malting, and that marked difference existed among cultivars and locations for the degraded proportion.

*Table 2.* Mean values for wort viscosity, malt friability, and β-glucans in barley grain, β-glucans in malt, and β-glucanase activity in malt

| Cultivar/ Location/ Seasonal type | Viscosity (mPas) | Friability (%) | β-Glucans in barley (%) | β-Glucans in malt (%) | β-Glucanase activity in malt (U $kg^{-1}$) |
|---|---|---|---|---|---|
| Zlatko | 1.845 $^{a*}$ | 53.20 $^{c}$ | 5.05 $^{ab}$ | 1.60 $^{b}$ | 381.5 $^{b}$ |
| Barun | 1.793 $^{a}$ | 50.80 $^{c}$ | 5.15 $^{ab}$ | 2.00 $^{a}$ | 421.5 $^{b}$ |
| Vanessa | 1.593 $^{b}$ | 68.40 $^{b}$ | 3.80 $^{b}$ | 1.32 $^{bc}$ | 392.0 $^{b}$ |
| Fran | 1.513 $^{b}$ | 81.85 $^{a}$ | 4.90 $^{ab}$ | 0.97 $^{cd}$ | 467.0 $^{b}$ |
| Matej | 1.539 $^{b}$ | 73.15 $^{ab}$ | 5.30 $^{ab}$ | 1.26 $^{bc}$ | 381.5 $^{b}$ |
| Scarlett | 1.483 $^{b}$ | 82.60 $^{a}$ | 5.95 $^{a}$ | 0.63 $^{d}$ | 601.0 $^{a}$ |
| Osijek | 1.633 $^{a}$ | 70.33 $^{a}$ | 4.85 $^{a}$ | 1.29 $^{a}$ | 464.8 $^{a}$ |
| Nova Gradiška | 1.622 $^{a}$ | 66.33 $^{a}$ | 5.20 $^{a}$ | 1.30 $^{a}$ | 416.7 $^{a}$ |
| Winter | 1.743 $^{a}$ | 57.47 $^{b}$ | 4.67 $^{a}$ | 1.64 $^{a}$ | 398.3 $^{b}$ |
| Spring | 1.512 $^{b}$ | 79.20 $^{a}$ | 5.38 $^{a}$ | 0.95 $^{b}$ | 483.2 $^{a}$ |
| Average | 1.627 | 68.33 | 5.03 | 1.29 | 440.8 |

*: significant difference ($P \leq 0.05$) is indicated by different letters

In our study, spring cultivar Scarlett had the highest activity malt β-glucanase, being significantly higher than the other 5 cultivars (Table 2). However, no difference was found among locations Osijek and Nova Gradiška. It may be suggested that variation of β-glucanase activity is more attributable to the genotype and seasonal type than to the location. Moreover, malt β-glucan content was not only dependent on the original level in grains, but also on malt β-glucanase activity. The lowest malt β-glucan content in Scarlett can be attributed to its higher β-glucanase activity, though it had the highest grain β-glucan content among 6 cultivars (Table 2). These initial results suggested that the ability to develop high levels of β-glucanase activity during malting is more important than lower grain β-glucan content in breeding barley for malting. This is in correlation with findings of WANG and co-workers (2004). Results from their study showed that barley grains had low but detectable β-glucanase,

which had a nearly 8-fold increase in malt, and that a greater cultivar and environmental variation was found in β-glucanase activity than in β-glucan content. BARBER and co-workers (1994) found that higher levels of β-glucanase activity were produced during malting by grain of cultivars with good malting quality. RIMSTEN and co-workers (2002) also showed that there were great effects of malting on β-glucanase in barley.

The results of correlation analysis between barley and malt β-glucan contents, β-glucanase activity, and six malt quality parameters are shown in Table 3. Barley grain β-glucan content had no significant correlations with other malt qualities. On the other hand, lower malt β-glucan content was highly significantly correlated with lower viscosity and fine/coarse extract difference and higher friability, which indicates its significance in the determination of malt quality. Higher malt β-glucanase activity was closely associated with lower malt β-glucan content and wort viscosity, and higher Kolbach index and friability, suggesting its positive function in malt modification. STUART and co-workers (1988) concluded that malt β-glucan was negatively and significantly correlated with malt extract. Results of WANG and co-workers (2004) showed that four malt quality parameters, including malt extract, Kolbach index, diastatic power, and viscosity, are all highly significantly correlated with malt β-glucan content, and their association with grain β-glucan content is relatively weak.

*Table 3.* Correlation coefficients between barley grain and malt quality parameters

| | Barley β-glucans | Malt β-glucans | Malt β-glucanase | Malt extract | Kolbach index | Diastatic power | Fine/ Coarse difference | Viscosity | Friability |
|---|---|---|---|---|---|---|---|---|---|
| Malt β-glucans | –0.245 | | | | | | | | |
| Malt β-glucanase | 0.339 | –0.660* | | | | | | | |
| Malt extract | –0.454 | –0.480 | 0.418 | | | | | | |
| Kolbach index | 0.048 | –0.295 | 0.642* | 0.224 | | | | | |
| Diastatic power | –0.223 | 0.237 | 0.083 | 0.114 | 0.398 | | | | |
| Fine/Coarse difference | –0.109 | 0.826* | –0.481 | –0.445 | –0.102 | 0.220 | | | |
| Viscosity | –0.105 | 0.850* | –0.564* | –0.443 | –0.275 | 0.329 | 0.914* | | |
| Friability | 0.119 | –0.930* | 0.633* | 0.586* | 0.217 | –0.387 | –0.862* | –0.934* | |
| Grain protein | 0.460 | –0.054 | 0.116 | –0.456 | –0.212 | 0.087 | –0.060 | –0.008 | –0.010 |

*: $P \leq 0.05$

A principal component analysis (PCA) biplot (Fig. 1) was constructed to provide an overview of spatial interrelationships between the cultivars, but it also helps to detect and interpret sample patterns. Two new variables were obtained, factor 1, which explains 46.3% of the data variance, and factor 2, which explains approximately 19.8% of the data variance. Thus, the dimensionality of the results was reduced from ten variables to two principal components, and the sum of the data variance explained by these two components is approximately 66.1%. Based on obtained malt quality attributes under cultivar and

environment influences, it is possible to distinguish clearly separated the group of spring cultivars (Scarlett, Fran, Matej) from the group of winter cultivars (Zlatko, Barun). Winter cultivar Vanessa's position in the plot is more comparable to those of the spring group cultivars due to its better malting performance.

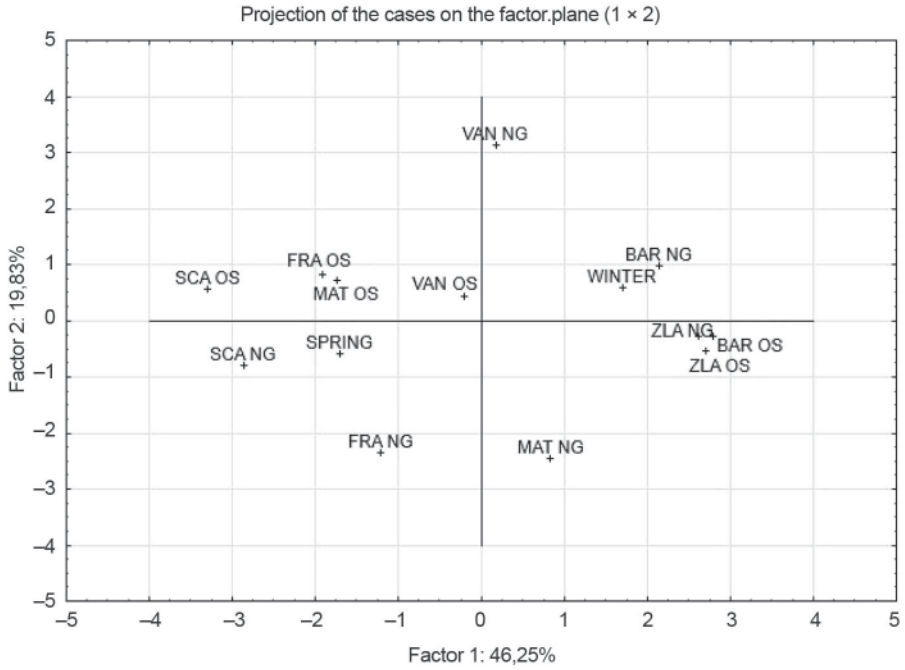


*Fig. 1.* PCA biplot over the barley cultivars and the winter and spring type groups. Abbreviations are as followed: ZLA: Zlatko; BAR: Barun; VAN: Vanessa; FRA: Fran; MAT: Matej; SCA: Scarlett; OS: Osijek; NG: Nova Gradiška

## 3. Conclusions

Cultivars in this study were selected to give a range of malting characteristics from both winter- and spring-sown barley materials. Malting performance showed that modification of barley grain endosperm during malting is closely correlated with β-glucan content in malt and β-glucanase activity, and that malt β-glucan content was more dependent on malt β-glucanase activity than the original level of β-glucan in grains. The obtained results indicate variability in malt β-glucan content and β-glucanase activity among analysed cultivars, which suggests that this variability should be explored for better understanding and closer specification of winter and spring malting barley attributes.

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