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The changes of β-glucan content and β-glucanase activity in barley before and after malting and their relationships to malt qualities

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Abstract

The cultivar and environmental variations of β-glucan content and β-glucanase activity in grains and malt were investigated in 8 barley cultivars grown at 7 locations. On average, for all cultivars and locations, approximately 80% of β-glucan present in grains was degraded after malting, but there was great variation among both cultivars and locations. β-glucanase activity was much lower but detectable in grains, and it dramatically increased after malting. The cultivar and environmental variations of β-glucan content were much higher in malt than in grains, and malt β-glucan content was more dependent on malt β-glucanase activity than the original level of β-glucan in grains. The correlation analysis of β-glucan content, β-glucanase activity and four malt quality parameters (Kolbach index, diastatic power, viscosity and malt extract) showed that malt-glucan content was highly significantly correlated with all quality parameters, while the association between grain β-glucan content and these quality parameters was relatively weak. Malt β-glucan content was negatively and highly significantly correlated with malt β-glucanase activity, and positively and significantly correlated with grain β-glucan content. It was also found that there was a positive and significant correlation between grain and malt β-glucanase activity.

Keywords: Barley (*Hordeum vulgare* L.); β-Glucan; β-Glucanase; Cultivar; Environment

1. Introduction

The changes of both $(1\rightarrow3,1\rightarrow4)$-β-D-glucan (referred to as β-glucan) content and $(1\rightarrow3,1\rightarrow4)$-β-D-glucan, 4-glucanohydrolase (referred to as β-glucanase, E.C.3.2.1.73) activity in barley during malting are interesting to breeders and malt producers, as both of them are closely associated with malt yield and quality. β-glucanase is a cell wall polysaccharide, which accounts for approximately 70% (w/w) of the endosperm cell in barley (Forrest & Wainwright, 1977; Jeraci & Lewis, 1989). In the brewing industry, a high content of β-glucan in barley may lead to insufficient degradation of cell walls, which in turn hinders the diffusion of germination enzymes and the mobilization of kernel reserves, and hence reduces malt extract. Residual β-glucan may also lead to highly viscous wort, giving rise to a filtration problem in the brewery, and it may participate in maturing of beer, causing chill haze (Bamforth, 1982; Palmer, 1975). 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 (Etokakpan, 1993). 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.

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Many reports show that there is a great genetic and environmental variation for both β-glucan content (Lehtonen & Aikasalo, 1987; Narasinhalu et al., 1994; Zhang, Chen, Wang, & Ding, 2001) and β-glucanase activity (Bamforth & Martin, 1983; Barber, Jackson, & Smith, 1994; Henry & Johnston, 1991; Loi, Barton, & Fincher, 1987) in barley. Thus, malt barley breeders face the problem, which should be the priority in evaluation and selection of breeding lines, β-glucan content or β-glucanase activity? For a long time, β-glucan content in grains has been the major objective to be improved in malt barley breeding due to its visually determinant influence on malting performance and easy assay. In the recent years, the impact of β-glucanase on malt quality has become more and more noted. According to Stuart, Loi, and Fincher (1988), in cultivars grown over a range of Australian environments, low β-glucan at the end of malting was the most important indicator of malting quality. In contrast to Morgan's (1977) emphasis on selection for low levels of β-glucan in the grains, they found that it was necessary to combine both relatively low grain β-glucan and the ability to develop high levels of β-glucanase. They concluded that, under Australian conditions, the level of β-glucan in the grains was not a good indicator of the level in malt and that, as β-glucanase showed large environmental variation, selection for stable expression would be advantageous. In the previous study (Zhang, Wang, & Chen, 2002), we found that most malt barley cultivars in southern China did not have higher β-glucan content than imported barleys from Australia or Canada, while malting performance of these Chinese barleys was relatively inferior. It was reported that higher levels of β-glucanase were produced during malting by grain of cultivars with good malting quality, thus emphasizing the importance of the breakdown of cell walls in malting (Barber et al., 1994).

β-Glucanase is predominantly synthesized in the aleurone and scutellum of germinated barley and secreted into the endosperm cells (Luchsinge, Cochrane, & Kneen, 1960; MacGregor, Dushnicky, Schroeder, & Balance, 1994; Mcfadden, Ahluwalia, Clarke, & Fincher, 1998). Thus, during malting, β-glucan content shows substantial decline, accompanied by increase in β-glucanase activity. Moreover, in matured barley seeds, a slight amount of β-glucanase can also be detected. However, little research has been reported on the relationship between seed and malt β-glucanase and the genetic and environmental variation of changes in β-glucan content and β-glucanase activity during malting. In this study, we investigate the cultivar and environmental variation of β-glucan content and β-glucanase activity, both in barley seeds and malt, by using 8 barley cultivars grown at 7 locations in China, and simultaneously analyze the relationship between these measurements and malt quality traits.

2. Materials and methods

2.1. Plant materials

In the 2000–2001 barley-growing season, eight two-rowed and winter barley cultivars, currently being widely planted in southern China (see Table 1), were grown at seven locations with different ecological conditions. Each cultivar consisted of 10 2 m-length lines. At maturity, 8 lines in the medium of each cultivar were harvested and the grains were used as the sample for assay.

2.2. Preparation of malt and quality analysis

Grain samples (200 g of each cultivar) were micromalted in a Phoenix System Micro-malting Apparatus using the regime: 6 h steep, 14 h air-rest, 8 h steep, 14 h air-rest and 4 h steep followed by 96 h modification – all at 15 °C. The malts were then kilned at 65 °C for 24 h, derooted and milled using a Tecator Cyclone mill fitted with a 0.5 mm screen. The malt quality parameters, extract, Kolbach index, viscosity and diastatic power (DP), were determined according to Analytica EBC Official Methods (European Brewery Convention, 1975).

Table 1
Grain β-glucan content (%) for 8 barley cultivars grown at 7 locations

	Hangzhou	Nanchong	Yancheng	Zhengzhou	Putian	Taian	Jingzhou	Mean	CV (%)
Zheyuan 18	3.35	3.59	3.80	3.76	3.10	3.47	3.19	3.47abc[A]	7.78
ZAU 3	3.52	2.56	3.73	3.98	3.77	2.99	3.23	3.40bc	14.7
Xiumai 3	3.47	3.82	3.88	4.15	3.78	3.75	3.42	3.75a	6.67
Zhepi 4	2.48	3.31	3.55	3.43	3.58	3.09	3.04	3.21cd	11.8
Daner Barley	2.89	3.68	2.99	3.83	3.28	3.07	3.17	3.27bcd	10.7
Gangpi 1	2.84	3.17	2.87	3.43	3.13	2.73	2.56	2.96d	10.1
Suyinmai 2	3.11	4.02	3.87	3.67	4.14	3.23	3.13	3.60ab	12.2
Yanyin 1	2.92	3.59	3.69	3.62	4.14	2.72	3.11	3.40bc	14.7
Mean	3.07b	3.48a	3.55a	3.73a	3.62a	3.13b	3.11b	3.38	11.1
CV (%)	11.7	12.9	11.3	6.70	11.6	11.2	8.04	10.5	

[A] The values within a column or a line followed by different letters are significantly different at 95% probability.

2.3. β-Glucanase and β-glucan assay

β-Glucanase was assayed by the method of McCleary and Shameer (1987) using Megazyme kits (Megazyme Ltd. Ireland). A unit (U) of activity is defined as the release of 1 μmol/min of reducing sugar at 30 °C, pH 4.6. Total β-glucan content in grains and malts was analysed according to the McCleary and Glennie-Homes (1985), using a commercial kit (Megazyme Ltd. Ireland), and presented on the basis of dry mass with 12% of moisture content.

3. Results

3.1. β-Glucan content in grains and malt

β-Glucan contents in grains and malt, for 8 cultivars grown at 7 locations, are shown in Tables 1 and 2, respectively. There were significant differences among cultivars and locations in β-glucan content of both grains and malt (germinated grains). The total mean β-glucan content for grains, across all locations and cultivars, was 3.38%, ranging from 2.96% for Ganpi 1 to 3.75% for Xiumai 3, in terms of 8 cultivars averaged over 7 locations and from 3.07% in Hangzhou to 3.73% in Zhengzhou, in terms of 7 locations averaged over 8 cultivars. It can be also seen that the coefficient of variation (CV) of grain β-glucan content varied greatly in cultivars and locations. It ranged from 6.67% for Xiumai 3 to 14.7% for Yanyin 1 and ZAU 3 among cultivars, and from 6.70% at Zhengzhou to 12.9% at Nanchong among locations. Xiumai 3 and Zhengzhou had the highest grain β-glucan content with the least variation among cultivars and locations, respectively.

After malting, the β-glucan content in malt was dramatically reduced. The β-glucan content for 8 barley cultivars in different locations ranged from 0.58% for Daner Barley to 0.94% for ZAU 3. For locations, it varied from 0.55% in Hangzhou to 1.03% in Putian. The total mean β-glucan content in malt, across all cultivars and locations, was 0.73%, being 21.8% of the total content in grains. This means that approximately 80% of β-glucan present in grains is degraded during malting. However, there was a great difference among both cultivars and locations for the degraded proportion. For instance, only 16.8% of grain β-glucan content remained in malt for Xiumei 3, averaged over all locations, correspondingly it was 26.8% and 27.6% for Zheyuan 18 and ZAU 3, respectively. There was a greater difference among locations. Thus the degraded proportion of β-glucan in malt varied from 71.6% at Putian to 83.1% at Nanchong. In addition, it can be seen from Tables 1 and 2 that the CV of both cultivar and location in β-glucan content was much 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, Xuimei 3 had the lowest β-glucan content in malt, even though it had the highest content in grains.

3.2. β-Glucanase activity in grains and malt

Low levels of barley β-glucanase activity were detected in grains (Table 3). On an average, in 8 cultivars, mean β-glucanase activity varied from 38.7 U/kg at Zhengzhou to 57.2 U/kg at Hangzhou, and a significant difference existed among locations. However, no difference was found among cultivars. After malting, β-glucanase activity was dramatically increased in malt

Table 2
Malt β-glucan content (above line, %) and its relative proportion to grains (below line, %) for 8 barley cultivars grown at 7 locations

	Hangzhou	Nanchong	Yancheng	Zhengzhou	Putian	Taian	Jingzhou	Mean	CV (%)
Zheyuan 18	0.94	0.64	0.93	0.83	1.21	1.03	0.90	0.93a[A]	19.4
	28.0	17.8	24.5	22.1	39.0	29.7	28.2	26.8	
ZAU 3	0.75	0.45	1.02	0.92	1.44	1.25	0.76	0.94a	35.1
	21.3	17.6	27.4	23.1	38.2	41.8	23.5	27.7	
Xiumai 3	0.33	0.49	0.63	0.83	0.82	0.75	0.57	0.63b	28.6
	9.51	12.8	16.2	20.0	21.7	20.0	16.7	16.8	
Zhepi 4	0.46	0.85	0.78	0.76	1.04	0.66	0.50	0.72b	27.8
	18.6	25.7	22.0	22.2	29.1	21.4	16.4	22.4	
Daner Barley	0.39	0.77	0.52	0.41	0.97	0.55	0.44	0.58b	37.9
	13.5	20.9	17.4	10.7	29.6	17.9	13.9	17.7	
Gangpi 1	0.52	0.52	0.76	0.88	1.13	0.62	0.65	0.73b	30.1
	18.3	16.4	26.5	25.7	36.1	22.7	25.4	20.3	
Suyinmai 2	0.46	0.41	0.7	0.99	0.81	0.78	0.66	0.69b	29.0
	14.8	10.2	18.1	27.0	19.6	24.2	21.1	19.2	
Yanyin 1	0.54	0.58	0.77	0.68	0.85	0.39	0.56	0.62b	24.2
	18.5	16.2	20.9	18.8	20.5	14.3	18.0	18.2	
Mean	0.55c	0.59c	0.76b	0.79b	1.03a	0.75b	0.63bc	0.73	
	17.9	17.0	21.4	21.2	28.5	24.0	20.3	21.6	29.0
CV (%)	36.4	27.1	21.1	22.8	21.4	36.0	23.8	26.9	

[A] The values within a column or a line followed by different letters are significantly different at 95% probability.

Table 3
Grain β-glucanase activity (U/kg) for 8 barley cultivars grown at 7 locations

	Hangzhou	Nanchong	Yancheng	Zhengzhou	Putian	Taian	Jingzhou	Mean	CV (%)
Zheyuan 18	64.6	41.5	38.2	41.9	64.6	39.8	49.8	48.6a[A]	23.7
ZAU 3	72.9	40.7	44.0	39.8	56.4	37.8	27.4	45.6a	32.5
Xiumai 3	62.2	50.2	48.1	27.4	72.1	41.9	46.4	49.8a	30.8
Zhepi 4	44.8	44.0	33.2	44.0	48.9	48.5	38.2	43.1a	13.1
Daner Barley	53.1	39.8	50.6	26.2	69.6	41.9	66.7	49.7a	30.8
Gangpi 1	48.1	35.7	58.4	25.8	48.9	48.1	29.5	42.1a	28.3
Suyinmai 2	49.8	37.4	25.8	52.2	53.1	31.6	29.5	39.9a	29.1
Yanyin 1	62.6	27.0	48.1	52.6	50.2	38.2	31.6	44.3a	28.4
Mean	57.2a	39.5b	43.3b	38.7b	58.0a	41.0b	39.9b	45.4	27.1
CV (%)	17.0	17.0	24.1	28.7	16.4	13.6	34.2	21.6	

[A] The values within a column or a line followed by different letters are significantly different at 95% probability.

Table 4
Malt β-glucanase activity (U/kg) for 8 barley cultivars grown at 7 locations

	Hangzhou	Nanchong	Yancheng	Zhengzhou	Putian	Taian	Jingzhou	Mean	CV (%)
Zheyuan 18	306	477	303	314	408	263	365	348.8b[A]	21.3
ZAU 3	477	314	219	278	368	226	310	313.1b	28.5
Xiumai 3	408	498	404	419	579	424	435	452	14.2
Zhepi 4	317	336	300	302	383	311	393	334	11.5
Daner Barley	438	420	440	434	503	571	624	490a	16.3
Gangpi 1	342	441	335	380	439	342	306	369b	14.3
Suyinmai 2	353	371	314	303	292	314	270	317b	11.1
Yanyin 1	368	384	329	299	376	317	377	350b	9.71
Mean	376abc	405ab	330c	341c	418a	346bc	385abc	372	16.0
CV (%)	16.0	16.1	20.4	17.7	21.2	31.1	28.7	21.6	

[A] The values within a column or a line followed by different letters are significantly different at 95% probability.

(Table 4). The total mean β-glucanase activity across all cultivars and locations was 372 U/kg, being more than 8-fold larger than that in grains. There were significant differences among both cultivars and locations. Daner Barley and Xiumai 3 had the highest activity, being significantly higher than other 6 cultivars. Among locations, Putian and Yancheng ranked first and last in the β-glucanase activity, respectively. In addition, the CV of 8 cultivars grown in various locations ranged from 9.71% for Yanyin 1 to 28.5% for ZAU 3, with a total mean of 15.8%. Correspondingly, CV for 7 locations ranged from 16.0% at Hangzhou to 31.1% at Taian, with a total mean of 21.6%. It may be suggested that variation of β-glucanase activity is more attributable to the environment than to the genotype.

3.3. The relationships between β-glucan content, β-glucanase activity and malt qualities

The results of correlation analysis between β-glucan content, β-glucanase activity and four malt qualities are shown in Table 5. Higher grain β-glucan content was associated with higher malt β-glucan content ($P < 0.05$)

Table 5
Correlation coefficients between β-glucan content, β-glucanase activity and four malt quality parameters

	Grain β-glucanase	Grain β-glucan	Malt β-glucan	Malt β-glucanase	Kolbach index	Diastatic power	Viscosity
Grain β-glucan	−0.0524						
Malt β-glucan	0.1363	**0.2883***					
Malt β-glucanase	**0.3822****	0.0513	**−0.2801***				
Kolbach index	0.0210	−0.1382	**−0.4737****	**0.3994****			
Diastatic power	0.0431	0.1228	**0.3630****	−0.1949	**−0.6200****		
Viscosity	−0.2045	**0.2878***	**0.3932****	**−0.4102****	**−0.6933****	**0.4153****	
Malt extract	−0.1418	−0.1476	**−0.4951****	0.1679	**0.8589****	**−0.6042****	**−0.5678****

$^{*}P < 0.05$.
$^{**}P < 0.01$.

and viscosity ($P < 0.05$), but had no significant correlation with other malt qualities. On the other hand, malt β-glucan content was highly significantly correlated with all four qualities, indicating its significance in the determination of malt quality. Higher malt β-glucanase activity was closely associated with lower malt β-glucan content ($P < 0.05$) and wort viscosity ($P < 0.01$), and higher Kolbach index ($P < 0.01$), suggesting its positive function in malt performance. Moreover, it was also noted that there was a significantly positive correlation between grain and malt β-glucanase activities.

4. Discussion

Change of β-glucan content during malting has been reported (Bamforth & Martin, 1983; Ellis et al., 1997; Stuart et al., 1988). Ellis et al. (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 et al. (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. In our study, on average for all cultivars and locations, approximately 80% of β-glucan present in grains was degraded during malting. However, a marked difference existed among cultivars and locations for the degraded proportion.

It has been reported that ungerminated barley grains are very low in β-glucanase activity (Barber et al., 1994; Ellis et al., 1997). Our results showed that grains had low but detectable β-glucanase, which had a nearly 8-fold increase in malt. Barber et al. (1994) found that higher levels of β-glucanase activity were produced during malting by grain of cultivars with good malting quality. Rimsten et al. (2002) also showed that there were great effects of malting on β-glucanase in barley grain. In our study, a greater cultivar and environmental variation was found in β-glucanase activity than in β-glucan content. Moreover, malt β-glucan content was not only dependent on the original level in grains, but also, to a greater extent, on malt β-glucanase activity. The lower malt β-glucan content in Xiumei 3 can be attributed to its higher β-glucanase activity, though it had the highest grain β-glucan content among 8 cultivars. It may be suggested that the ability to develop high levels of β-glucanase during malting is more important than a lower grain β-glucan content in malt barley breeding.

Malting performance is closely correlated with β-glucan content and β-glucanase activity (Ellis et al., 1997; Henry, 1989; Knuckles & Chiu, 1999). Ellis et al. (1997) showed that β-glucanase activity in germinated grains was negatively and significantly correlated with its unextractable β-glucan content. Stuart et al. (1988) concluded that malt β-glucan was negatively and significantly correlated with malt extract, and that grain β-glucanase activity was positively and significantly correlated with malt extract. Our results show that four malt quality parameters are all highly significantly correlated with malt β-glucan content, and their association with grain β-glucan content is relatively weak. Malt β-glucan content is more dependent on malt β-glucanase activity than grain β-glucan content. Moreover, a positively significant correlation exists between malt and grain β-glucanase activity. This suggests that malt β-glucanase activity can be preliminarily estimated by measuring grain β-glucanase activity. Such estimation is imperative in barley breeding programmes, as large amounts of seeds and high costs are involved.

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