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Studies on water-extractable arabinoxylans during malting and brewing

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Abstract

The most important non-starch polysaccharides in barley grain are β-glucan and arabinoxylans. Arabinoxylans are partially water-extractable, high-molecular-weight polymers that contribute to viscosity and membrane filterability. Changes to arabinoxylans content and *endo*-xylanase activity during germination and kilning were studied. This paper was aimed at determining the composition of total arabinoxylans and water-extractable arabinoxylans in malt and observing their changes during brewing. The water-extractable and total arabinoxylans contents of six barley malts and wheat malt varied in the range of 0.42–0.98% and 3.84–6.90%, respectively. Their L-arabinose-to-D-xylose ratio ranged between 0.49–0.59 (total arabinoxylans) and 0.60–0.85 (water-extractable arabinoxylans). Contents of arabinoxylans in worts mainly originated from the soluble part of arabinoxylans in malt. The use of rice adjunct decreased arabinoxylans contents of wort and beer. The correlation between arabinoxylans and viscosity was investigated. Results showed that water-extractable arabinoxylans in wort have positive and higher correlation value with wort viscosity than those of β-glucan in wort, and both water-extractable arabinoxylans and β-glucan in beer have significant correlation ($p < 0.01$) with beer viscosity.

Keywords: Arabinoxylans; Malt; Wort; Beer

1. Introduction

The major constituents of the walls of the starchy endosperm in barley grain are the β-glucan and arabinoxylans (AX). In under-modified malts, the fact that arabinoxylans cannot be degraded sufficiently, may cause many problems such as low extract yield, high wort viscosity, decrease of filtration rate, and haze formation in brewing (Coote & Kirsop, 1976).

AX consists of a linear back bone of (1 → 4)-β-D-xylopyranosyl units, to which α-L-arabinofuranosyl substituents are attached through O-2, O-3, or O-2,3. One of the unique features of AX is the presence of ferulic acid covalently linked via an ester linkage to C(O)-5 of the arabinose residue. Furthermore, ferulic acid dimers are detected at a rather high proportion in malt AX. This suggested that water-extractable arabinoxylans (WEAX) might be partially cross-linked with the possibility of causing filtration problems (Figueroa & Rouau, 1998; Izydorcyk, Biliaderis, & Bushuk, 1990).

Historically, reduced beer filtration efficiency has been mainly attributed to β-glucan in the brewing process. β-glucan may increase the viscosity of beer by forming gels, consisting primarily of high-molecular-weight β-glucan molecules (Home, Stenholm, Wilhelmson, & Autio, 1999). Recently, it was found that other large molecules such as AX, proteins and polyphenols were

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also associated with reduced beer filtration, in particular micro-filtration. In fact, it has been reported that the amount of AX in commercial beer is approximately 10 times greater than that of β-glucan (Schwarz & Han, 1995). Paul's (2002) research indicated that the effects of AX on viscosity and filterability were equivalently important to those of β-glucan. Stewart, Hawthorne, and Evans (1998) found that viscosity and membrane filterability of the pilot-brewed beer were correlated with AX content, whereasβ-glucan was only correlated with viscosity.

The enzymes that degrade AX are often produced late in the germination process (Banik, Li, Langridge, & Fincher, 1997), and high levels of AX can survive through the brewing into the final beer (Coote & Kirsop, 1976). Some AX are solubilized from the cell walls but are not extensively degraded by endogenous enzymes during malting (Voragen, Schols, Marius, Rombouts, & Angelino, 1987). Structural features of arabinoxylans from barley, malt and wort have been extensively reported (Han, 2000; Viëtor, Angelino, & Voragen, 1992; Viëtor, Voragen, & Angelino, 1993). Malt extracts can contain high levels of AX and attendant difficulties associated with the filtration of viscous extracts may significantly deteriorate the performance of the brewing processes (Bamforth, 1985). These brewing problems were more pronounced when wheat malt was used as adjunct for its higher AX content and higher molecular weight.

To the best of our knowledge, no systematic study on AX degradation during malting and brewing has been reported. In this paper, the effects of germination and kilning processes on AX degradation and *endo*-xylanase activity, the contents of AX in malt, wort and beer, and the correlation between AX and viscosity, were investigated.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical grade. A kit of neutral sugars and 1-methylimidazole were purchased from Sigma Co., Ltd. Congo red was purchased from Fluka Co., Ltd.

2.2. Barley malt samples

Five barley malt samples were obtained from commercial malt factories. Harrington and Maltcafe were from Canada, Stirling was from Australia, KA4B-1, KA4B-2, and wheat malt were from China. Schooner (Australia) variety of barley was malted and studied for changes of AX content and *endo*-xylanase activity during germination and kilning.

2.3. Micromalting, mashing and brewing

Micromalting was carried out in a microprocessor-controlled facility where steeping, germination and kilning were carried out in a single unit. Schooner barley was germinated for 4 days. The samples were kilned for 22 h using a stepwise temperature cycle, starting at 45 °C and finishing with curing at 82–84 °C. Test points were performed at the end of each stepwise temperature program.

Worts were prepared in duplicate. About 500 g of barley malt were used for preparing 100%-malt wort. About 350 g of barley malt and 150 g of rice were used for 70%-malt wort. The mashing-in temperature was at 50 °C and the final temperature was at 78 °C. After cooling, the wort was adjusted to 11 °C, and then it was fermented with yeast.

2.4. Malt, wort and beer analysis

The content of moisture and protein, and extract yield of the malts were analyzed by a grain analyzer (Foss Tecator Infratec 1229, Sweden). The viscosity of wort and beer was determined by a microviscometer (Hoppler, Germany).

2.5. Determination of endo-xylanase activity

Preparation of enzyme extracts based on the method of Sungurtas, Swanston, Davies, and McDougall (2004). Barley malts were homogenized in a mortar and pestle in ice-cold homogenisation buffer (50 mM sodium acetate pH 5.0 containing 250 mM NaCl, 0.1% (w/v) polyvinyl polypyrrolidine (PVPP, ISP Co., Ltd.)) The addition of NaCl and PVPP enhanced recovery of *endo*-xylanase ativity. The homogenate was filtered and then extracts were dialysed overnight against 10 mM sodium acetate pH 5.0. The dialysates were applied to determine *endo*-xylanase activity. Endo-xylanase activity was determined by DNS method, according to the method of Bailey, Biely, and Poutanen (1992).

2.6. Determination of β-glucan in wort and beer

Determination of β-glucan content with Congo red dye was modified with the method of Li, Yin, Gu, and Lu (1997). β-glucan samples (100 μl) were mixed with 3.0 ml of 100 mg/l Congo red dissolved in 0.1 mol/l (pH 9.0) glycine-NaOH buffer. Absorbance at 550 nm was measured with a spectrophometer and double deionized water (100 μl) was mixed with 3.0 ml of 100 mg/l Congo red as a blank. β-glucan concentrations in the range of 0–1000 mg/l at intervals of 100 mg/l were used to prepare calibration curves.

2.7. Determination of arabinoxylans in malt, wort and beer

Six barley malts were milled with a pilot mill (mode A, Miag, Braunschweig, Germany). AX and WEAX were estimated by gas chromatography (GC), with the composing monosaccharide residues after hydrolysis as the index. The details were outlined below.

For determination of total AX contents, barley malts milling fractions (0.10 g) were hydrolyzed with 5.0 ml 2 mol/l trifluoroacetic acid (90 min, 121 °C) (Cleemput, Roles, Van Oort, Grobet P.J, & Delcour, 1993). After cooling, the hydrolysates were filtered. For determination of WEAX contents, samples of the milling fractions (1.0 g) were extracted with deionized water (1:10, w/v). After shaking (2 h, 30 °C), suspensions were centrifuged at 3000*g* for 15 min, and then hydrolyzed for 90 min with 2 mol/l trifluoroacetic acid (121 °C). Alditol acetates were prepared based on the method of Egylst and Cummings (1984). Separation of the alditol acetates was with a Finnigan (GC–MS) chromatograph using a SP-2330 column (30 m × 0.25 mm). The temperatures of injection and detection (flame ionization detector) were 260 and 280 °C, respectively. Total AX and WEAX contents were calculated as 0.88 × (% arabinose + % xylose) (Hery, 1986).

Worts were centrifuged at 3000*g* for 10 min (Debyser, Derdelinckx, & Delcour, 1997). Beers were firstly filtered through a micromembrane filter (0.65 μm, Satorious, Germany), and then they were pretreated with the method similar to that of worts. After hydrolysis and derivatization, the alditol acetates of worts and beers were prepared. The AX contents of worts and beers were determined by gas chromatography.

3. Results and discussion

3.1. Changes of AX content and ***endo***-xylanase activity during germination and kilning

During the germination period, the content of total AX in barley grain partially decreased, while solubilized AX content increased (Fig. 1). After 96 h germination, the total AX content decreased from 6.34% (% of dry malt) to 5.16% and WEAX content increased from 0.12% to 0.37%. The maximum of *endo*-xylanase activity occurred after 72 h germination.

After 22 h kilning, a slight change was observed between total AX content and WEAX content (Fig. 2). Total AX content decreased from 5.16% to 4.68% and WEAX content increased from 0.37% to 0.44%. The final *endo*-xylanase activity decreased to half of the initial enzyme activity after germination.

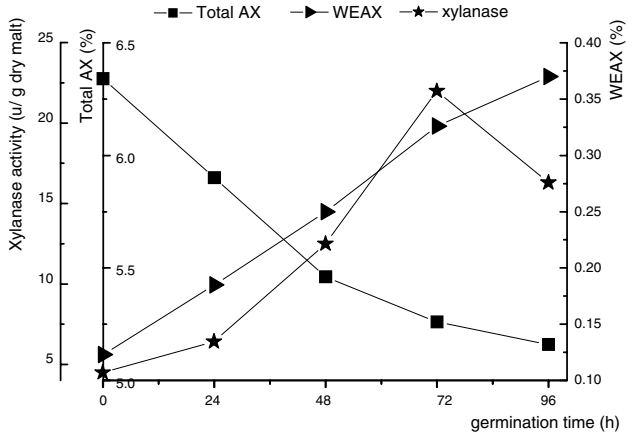

Fig. 1. Change patterns of AX and *endo*-xylanase activity during germination.

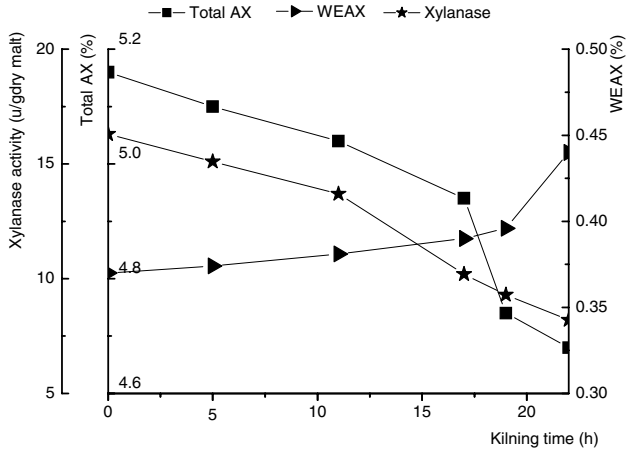

Fig. 2. Change patterns of AX and *endo*-xylanase activity during kilning.

3.2. Total AX content and WEAX content of malt

Analyses of six barley malts were shown in Table 1. All cultivars except Maltcafe and KA4B-1 had the higher extract value. Maltcafe and Schooner had the highest and the lowest protein content, respectively.

Table 1
Malt analyses

Malt	M	E	Pt	Ps	KI
Harrington	5.6	80.0	11.9	4.65	39.2
Maltcafe	5.5	79.5	12.0	4.83	40.1
Stirling	4.8	81.4	11.1	4.39	39.6
Schooner	5.0	80.6	10.5	4.32	41.0
KA4B-1	6.6	79.2	11.6	4.52	39.1
KA4B-2	6.4	80.3	11.7	4.71	40.3
Wheat malt	6.5	80.1	12.2	4.79	39.9

M, moisture content (%); E, extract content (% of dry malt); Pt, total protein content (% of dry malt); Ps, soluble protein content (% of dry malt); KI, Kolbach Index (Ps/Pt × 100).

Table 2
Total AX and WEAX (% of dry malt) of six barley malts and their arabinose to xylose ratio

Malt	Total AX (g/100 g dry malt)	Total AX (A/X)	WEAX (g/100 g dry malt)	WEAX (A/X)	WEAX/Total AX (%)
Harrington	3.84	0.53	0.42	0.75	10.9
Maltcafe	4.56	0.51	0.58	0.85	12.7
Stirling	5.40	0.59	0.70	0.83	13.0
Schooner	4.68	0.57	0.44	0.84	9.4
KA4B-1	3.64	0.49	0.50	0.72	13.7
KA4B-2	4.24	0.52	0.56	0.81	13.2
Wheat malt	6.90	0.51	0.98	0.60	14.2

Kolbach index gives the degree of solubility of barley protein during malting. Each sample gave a desired value (39.1–41.0).

The total AX content and WEAX content of barley malt and wheat malt are shown in Table 2. The total AX content varied from 3.84% to 6.90%, while the WEAX content ranged from 0.42% to 0.98%. Wheat malt had the highest contents for both the total AX and WEAX. The arabinose-to-xylose (A/X) ratio shows the degree of substitution of the xylan backbone by arabinose residues. In general, AX isolated from cell walls contain more arabinose than those isolated from the husk or aleurone (Izydorzyk & Biliaderis, 1995). It is reported that AX polymers with high intrinsic viscosity had low A/X ratios and there was a linear relation between the two parameters (Izydorzyk & Biliaderis, 1992). The A/X ratios of the total AX fraction and WEAX fraction were from 0.49 to 0.59 and 0.60 to 0.85, respectively. It showed that the WEAX fraction had a higher A/X ratio, which resulted in low intrinsic viscosity according to Izydorczyk's principle. The ratio of WEAX to total AX varied from 9.4% to 14.2%, which showed that most of AX in barley malt and wheat malt were insoluble.

3.3. WEAX content of wort

Table 3 shows that WEAX contents of 100%-malt wort were ranged from 946 to 1920 mg/l. About 100%-wheat malt wort had more WEAX content than those of barley malts. Stirling malt wort had the highest WEAX content (1791 mg/l) among 100%-barley malt worts. This may be explained by the relatively high WEAX content (0.70%) of Stirling malt. Generally, the WEAX contents in 100%-malt wort (0.50%–1.01%) were higher than the WEAX contents of malt (0.42–0.98%). It can be explained that *endo*-xylanase, still active at the beginning of the mashing process, renders AX more soluble (Debyser et al., 1997).

Adjuncts were used in brewhouse operation to increase extract yield and beer stability, and also to reduce production cost. Rice adjunct, at a 30% level, resulted in a decrease of WEAX and β-glucan contents, which corresponded to the relative low total AX (2.6%) and WEAX (0.15%) contents of rice (Table 4). The ratio of A/X in AX of 100%-malt wort was lower than that of 70%-malt wort. It may be explained that AX from rice seemed to consist of more highly branched xylan backbones than those from barley malt (Shibuya & Iwasaki, 1985).

3.4. WEAX content of beer

Tables 6 and 7 showed that the levels of WEAX were further decreased in beer brewing processes. The WEAX content of 100%-malt beer varied from 621 to 1171 mg/l. It would be explained that part of the high-molecular-weight AX was cut off by the micromembrance filtration. The WEAX contents of 100%-malt beers were generally greater than WEAX contents in 70%-malt beers .

3.5. Correlations among AX content, β-glucan content, and viscosity

The viscosity of wort and beer from the six barley malts varied considerably. Values ranged from 1.67 to 2.02 cp (Tables 3 and 5) and 1.47 to 1.56 cp (Tables 6 and 7), respectively. The relationship between the non-

Table 3
WEAX and β-glucan contents of wort (100% barley malt)

Malt	WEAX (mg/l)	WEAX (g/100 g dry malt)	A/X	β-glucan (mg/l)	Viscosity (cp)
Harrington	946	0.50	0.69	185.2	1.69
Maltcafe	1314	0.69	0.78	158.4	1.74
Stirling	1791	0.93	0.67	387.6	1.77
Schooner	1163	0.61	0.72	155.2	1.69
KA4B-1	1213	0.66	0.74	271.3	1.74
KA4B-2	1297	0.69	0.72	347.7	1.75
Wheat malt	1920	1.01	0.55	280.1	2.02

Table 4
Total AX and WEAX contents (% of dry rice) of adjunct rice

Pt (%)	M (%)	Total AX (g/100 g dry rice)	Total AX (A/X)	WEAX (g/100 g dry rice)	WEAX (A/X)
6.6	14.8	2.6	0.91	0.15	0.95

M, moisture content (%); Pt, total protein content (% of dry rice).

Table 5
WEAX and β-glucan contents of wort (70% barley malt + 30% rice)

Malt (70%) + rice (30%)	WEAX (mg/l)	WEAX (g/100 g dry mass)	A/X	β-glucan (mg/l)	Viscosity (cp)
Harrington + rice	804	0.44	0.75	140.1	1.68
Maltcafe + rice	1099	0.60	0.86	123.3	1.67
Stirling + rice	1680	0.91	0.69	251.0	1.75
Schooner + rice	962	0.52	0.70	114.3	1.67
KA4B-1 + rice	1107	0.61	0.81	231.1	1.73
KA4B-2 + rice	1189	0.65	0.77	245.3	1.70
Wheat malt + rice	1655	0.91	0.62	210.5	1.80

Table 6
WEAX and β-glucan contents of wort (100% barley malt)

Malt	WEAX (mg/l)	WEAX (g/100 g dry malt)	A/X	β-glucan (mg/l)	Viscosity (cp)
Harrington	621	0.32	0.73	117.5	1.49
Maltcafe	709	0.37	0.75	124.7	1.51
Stirling	932	0.48	0.65	189.4	1.52
Schooner	698	0.36	0.75	98.4	1.49
KA4B-1	788	0.42	0.70	157.8	1.52
KA4B-2	853	0.45	0.65	148.5	1.50
Wheat malt	1171	0.61	0.54	163.5	1.56

Table 7
WEAX and β-glucan contents of beer (70% barley malt + 30% rice)

Malt (70%) + rice (30%)	WEAX (mg/l)	WEAX (g/100 g dry mass)	A/X	β-glucan (mg/l)	Viscosity (cp)
Harrington + rice	535	0.30	0.77	97.1	1.48
Maltcafe + rice	624	0.38	0.80	88.2	1.47
Stirling + rice	810	0.46	0.65	155.2	1.51
Schooner + rice	580	0.32	0.72	61.6	1.48
KA4B-1 + rice	687	0.38	0.75	100.4	1.50
KA4B-2 + rice	734	0.40	0.71	116.5	1.48
Wheat malt + rice	987	0.55	0.61	117.1	1.52

Table 8
Correlations among AX content, β-glucan content and filtration viscosity

	WAX	WBG	WCP	BAX	BBG	BCP
WAX	1	0.590	0.790**	0.923**	0.757*	0.829**
WBG	–	1	0.520	0.690**	0.823**	0.589*
WCP	–	–	1	0.701*	0.590*	0.916**
BAX	–	–	–	1	0.729**	0.893**
BBG	–	–	–	–	1	0.738**
BCP	–	–	–	–	–	1

WAX, water-extractable arabinoxylans content of wort; WBG, β-glucan content of wort; WCP, viscosity of wort; BAX, water-extractable arabinoxylans content of beer; BBG, β-glucan content of beer; BCP, viscosity of beer

* Significant to $p < 0.05$.

** Significant to $p < 0.01$.

starch polysaccharides and the corresponding viscosities of wort and beer was investigated and correlation values for the seven malt samples are presented in Table 8. WEAX in wort gave positive and relative higher correlation value (0.790) with wort viscosity than that of β-glucan (0.520). Similarly, WEAX in wort gave positive and relative higher correlation value (0.829) with beer viscosity than that of β-glucan (0.589). Some filtration efficiency related brewing problems due to β-glucan in the past may actually be caused by the AX polymers. However, both of WEAX and β-glucan in beer showed significant correlation ($p < 0.01$) with beer viscosity, but WEAX in beer showed a slightly higher correlation value (0.893) with beer viscosity than with β-glucan (0.738). This reconfirmed that these non-starch polysaccharide polymers greatly affected the viscosity of beer.

4. Conclusions

During germination period, the content of total AX in barley grain partially decreased, while WEAX content increased. A slight change was observed between total AX content and WEAX content after kilning. The maximum development of *endo*-xylanase activity occurred after 72 h germination and remained active after kilning.

Most of the AX in barley malt was insoluble. WEAX content of 100%-malt wort (0.50–1.01%) was higher than WEAX content of malt (0.42–0.98%). The use of rice adjunct decreased WEAX content of wort and increased the ratio of A/X. WEAX contents of beers were lower than that of the worts. WEAX in wort gave positive and relative high correlation value with wort viscosity than with β-glucan. Both of WEAX and β-glucan in beer showed significant correlation ($p < 0.01$) with beer viscosity.

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