Activity of Arabinoxylan Hydrolyzing Enzymes during Mashing with Barley Malt or Barley Malt and Unmalted Wheat

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Pilot scale brews were prepared either with 100% barley malt (BM_{100}) or 60% barley malt and 40% unmalted wheat ($BM_{60}W_{40}$). Arabinoxylan and β-glucan hydrolyzing enzyme activities were determined during mashing using two temperature profiles. The measured enzymic activities increased for the BM_{100} and $BM_{60}W_{40}$ mashes in the early stages of mashing. The endoxylanase and α-L-arabinofuranosidase activities remained constant at 50 °C but rapidly decreased above 50 °C. At 72 °C, the endoxylanase and α-L-arabinofuranosidase activities were almost completely lost. The β-D-xylosidase activity only decreased slowly at 63 °C. The β-glucanase activity decreased rapidly at 50 °C and was completely lost after 15 min at 50 °C. From the xylose (Xyl) levels (a measure for arabinoxylan content) in the BM_{100} worts (1.28−1.33 g/L), a solubilization of 0.23−0.26% Xyl (% of cereal dry matter) during mashing was calculated. The Xyl levels in the $BM_{60}W_{40}$ worts (0.92−1.11 g/L) corresponded with a solubilization of 0.12 to 0.15% Xyl during mashing.

Keywords: *Endoxylanase; xylosidase; arabinofuranosidase; β-glucanase*

INTRODUCTION

Arabinoxylans are cereal cell wall components. They can be degraded by several enzymes (Figure 1). The 1,4-β-D-xylanhydrolases (EC 3.2.1.8), further referred to as endoxylanases, generate unsubtituted and substituted xylo-oligosaccharides. These oligomers are further degraded by β-D-xylosidases (EC 3.2.1.37), releasing β-D-xylose from the nonreducing end. The α-L-arabinose substituents from the main chain are liberated by α-L-arabinofuranosidases (EC 3.2.1.55). Finally, esterases (EC 3.1.1.6) release ferulic and *p*-coumaric acids esterified to *O*-5 of arabinofuranosyl residues.

Arabinoxylan degradation during the brewing process is desirable. Indeed, arabinoxylans have been associated with poor wort filterability (Barret et al., 1975) and haze formation (Coote and Kirsop, 1976). In this context, the use of endoxylanase reduces wort viscosity (Ducroo and Frelon, 1989; Viëtor et al., 1993).

Beers contain 0.5−4.2 g/L arabinoxylan with high levels for German wheat beers made with malted wheat (Schwarz and Han, 1995). In the case of Belgian wheat beers, made with 60% barley malt and 40% unmalted wheat ($BM_{60}W_{40}$), the arabinoxylan levels in wort seemed not to be higher than in case of worts from pure malt (BM_{100}) (Debyser et al., 1997a,b). However, there may be a difference in molecular weight of the arabinoxylans as the water-extractable arabinoxylans of barley malt (Debyser et al., 1997c) have a much lower molecular weight than those from wheat (Cleemput et al., 1993) and as the malt xylanolytic system is (to a certain degree) inactivated by wheat (Debyser et al., 1997b).

To the best of our knowledge, no systematic study of the activities of arabinoxylan degrading enzymes during mashing has been carried out. Therefore, the activity of the most important xylanolytic enzymes and also of β-glucanase was monitored during mashing both for BM_{100} and $BM_{60}W_{40}$. The molecular weight profiles of the arabinoxylans present in wort for the two different kind of beers were compared as well.

EXPERIMENTAL PROCEDURES

Chemicals. Specialty chemicals were azurine-cross-linked (AZCL) wheat arabinoxylan (Xylazyme Arabinoxylan tablets) and AZCL barley β-glucan from Megazyme (Bray, Ireland). *p*-Nitrophenyl α-L-arabinofuranoside, *p*-nitrophenyl β-D-xylopyranoside, and Trizma base (tris[(hydroxymethyl)amino]-methane, reagent grade) were obtained from Sigma (St. Louis, MO). Standard pullulans (P-82) were delivered by Showa Denko K. K. (Tokyo, Japan).

Barley Malt and Wheat. Barley malt (cv. Plaisant) was supplied by Cargill Malt Division (Herent, Belgium) and wheat (cv. Skirlou and cv. Soissons) by Sapsa-SES (Jodoigne, Belgium).

Brewing and Sampling. Brews (12 °P) were prepared in a pilot scale brewery (Delcour et al., 1984). The BM_{100} mashes were prepared with 12 kg of malt and 48 L of water. The $BM_{60}W_{40}$ mashes were made with 4.8 kg of wheat, 7.2 kg of malt, and 48 L of water. Two different temperature profiles were used in which temperature increases were a linear function of time. In temperature profile 1 (TP1), we started at 50 °C for 30 min followed by an increase to 63 °C in 40 min. After 30 min at 63 °C the temperature was raised to 72 °C in 30 min and was held at 72 °C for 15 min. In temperature profile 2 (TP2), we started mashing at 45 °C for 15 min and raised the temperature to 50 °C in 10 min. The mash was kept at 50 °C for 30 min followed by an increase to 63 °C in 40 min. After 45 min at 63 °C, the temperature was raised to 72 °C and kept for 15 min at 72 °C. Samples (20 mL) were taken from the mash at different times and immediately centrifuged (1500*g*; 5 min; 20 °C), frozen and stored (−18 °C) and are further referred to as mash samples. After an increase to 78 °C, the sweet wort was separated from the spent grains. The sweet wort of $BM_{60}W_{40}$ was boiled (60 min) and hopped (51 g/L; 9.7% α-acids) with addition of bitter orange peel powder

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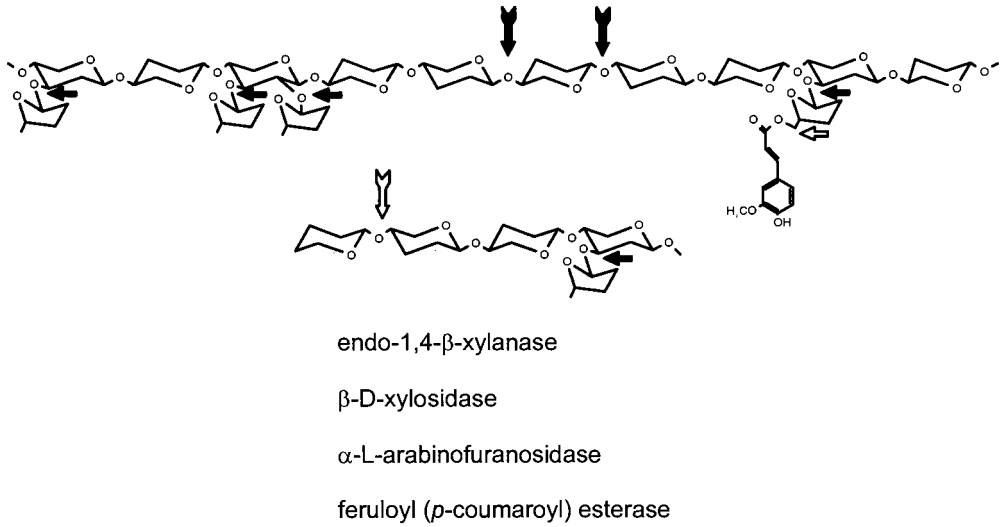

Figure 1. Sites of attack of enzymes hydrolyzing a hypothetical plant arabinoxylan.

(14 g/hL) and coriander (55 g/hL). After clarification, the hopped wort was cooled to 20 °C and aerated, and a top fermenting yeast (*Saccharomyces cerevisiae*) was added. After 7 days at 22 °C, samples were frozen and stored (−18 °C) (= green beer). The beer was then stored for 10 days at 13 °C for settlement of the yeast and maturation. Samples of the partially clarified beer were frozen and stored (−18 °C) (= lautered beer).

Measurement of endoxylanase (EC 3.2.1.8) activity. Endoxylanase activities of the mash samples were measured as described by Debyser et al. (1997a) with azurine-cross-linked wheat arabinoxylan (Megazyme, Bray, Ireland).

Measurement of *β*-D-Xylosidase (EC 3.2.1.37) and α-L-Arabinofuranosidase (EC 3.2.1.55) Activities. Nitrophenyl glycosides were used to measure *β*-D-xylosidase and α-L-arabinofuranosidase activities of the mash samples according to the procedure of Cleemput et al. (1995). One unit of activity was defined as the amount of enzyme that released 1 *μ*mol *p*-nitrophenol from the substrate per minute at 40 °C and pH 6.0.

Measurement of *β*-Glucanase (EC 3.2.1.73) Activity. Endo-*β*-glucanase activity was measured with azurine-cross-linked *β*-glucan (Megazyme, Bray, Ireland). The mash sample (1.0 mL) was incubated for 5 min at 40 °C, before adding an AZCL *β*-glucan tablet. The incubation was then continued for 10 min at 40 °C. The reaction was terminated by adding 2% (w/v) Trizma base (10.0 mL) and vigorous vortex stirring. After 5 min at room temperature, the tubes were shaken vigorously and the contents filtered through a Whatman no. 1 filter. The absorbance was measured at 590 nm against a control, which was prepared by incubating the extract without the substrate tablet for 10 min at 40 °C. The substrate tablet was then added after adding 2% (w/v) Trizma base to the extract. The activity was expressed as the difference in the absorbance at 590 nm between the sample and control and expressed per gram of dry matter of the starting materials (ΔA_{590}/g).

Determination of Levels of Xylose-Containing Material. Water extracts of malt and wheat were made as described previously (Debyser et al., 1997a). The water extracts of malt and wheat and the worts were thus hydrolyzed and the monosaccharides converted into alditol acetates. The latter were then analyzed by gas−liquid chromatography.

Determination of Polymeric *β*-Glucan. The polymeric *β*-glucan content was measured enzymically according to EBC method 3.13.1. (Analytica-EBC, 1998) using the *β*-glucazyme kit from Megazyme (Bray, Ireland).

All analyses described above were carried out at least in duplicate and the mean values are presented. The experimental error was calculated from the difference (in %) between the individual and the mean value.

Determination of Molecular Weight of Polymeric Arabinoxylans in Sweet Wort. Fractions (100 mL) of the worts were dialyzed (molecular weight cut off = 3,500; 48 h; 7 °C) to exclude monomeric and oligomeric carbohydrates and lyophilized. Redissolved wort material (40 *μ*L; 10 mg/mL) or molecular weight markers (20 *μ*L; 1 mg/mL) [pullulan (78.8×10^4 Da, 40.4×10^4 Da, 21.2×10^4 Da, 11.2×10^4 Da, 3.74×10^4 Da, 2.28×10^4 Da, 1.18×10^4 Da, and 0.59×10^4 Da); xylopentaose (678 Da), and xylose (150 Da)] were separated on a Shodex B-804 (Showa Denko K. K., Tokyo, Japan) gel permeation column (50 cm × 0.8 cm) by elution with 0.3% NaCl. Fractions (0.5 mL) were collected, lyophilized, and analyzed for pentosans using the orcinol method (Hashimoto et al., 1987). Each fraction was hydrolyzed at 100 °C (2 h) with 250 *μ*L of HCl (2 N). The hydrolysate was heated (100 °C) for 30 min after addition of 250 *μ*L of 0.1% $FeCl_3$ in concentrated HCl and 50 *μ*L of 1% orcinol in absolute ethanol. Absorbance was measured at 670 and 580 nm and the difference between the two plotted against the elution time.

RESULTS AND DISCUSSION

Activities of Arabinoxylan-Degrading Enzymes and *β*-Glucanases. Typical curves of the measured xylanolytic activities during mashing with $BM_{60}W_{40}$ and BM_{100} are shown in Figures 2 and 3, respectively. There was no obvious difference in profiles between mashing with cv. Skirlou and Soissons for any of the enzyme activities (results not shown).

Endoxylanase Activity. The recovered endoxylanase activity increased initially, from 0.25−0.31 ΔA_{590}/g to 0.36−0.42 ΔA_{590}/g for the $BM_{60}W_{40}$ mashes, and from 0.5−58 ΔA_{590}/g to 0.70−0.78 ΔA_{590}/g for the BM_{100} mashes, due to extraction and solubilization of the endoxylanases in the early stages of mashing. A starting temperature of 45 °C instead of 50 °C resulted in a longer time needed for maximal recovery of endoxylanase activity. The activity stabilized at 50 °C but decreased as soon as the mash temperature exceeded 50 °C. At 72 °C, the endoxylanase activity was almost completely lost. The maximum endoxylanase activities for the $BM_{60}W_{40}$ mashes were 12−24% lower than 60% of the values of the BM_{100} mashes. This was presumably caused by inhibition of the malt endoxylanases by wheat extractables. However, earlier results, suggesting stronger inhibition by cv. Skirlou than by cv. Soissons, could not be confirmed by the present brewing trials (Debyser et al., 1997b).

β-D-Xylosidase Activity. The *β*-D-xylosidase activity also increased in the early stages of mashing from 0.077−0.112 U/g to 0.172−0.182 U/g for the $BM_{60}W_{40}$

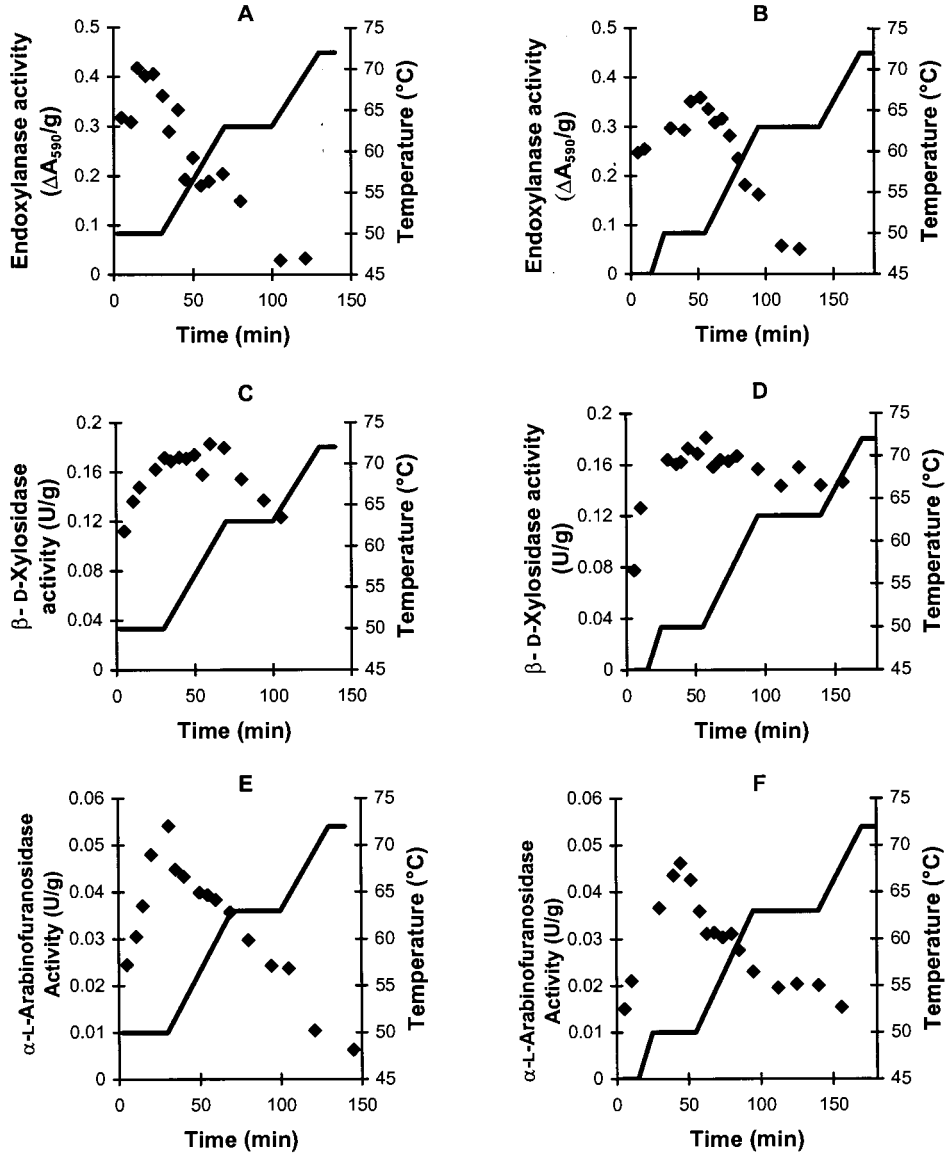

Figure 2. Arabinoxylan hydrolyzing activities (◆) during brewing with 60% barley malt and 40% wheat. Typical profiles (—) are shown for temperature profile 1 (A, C, and E) and temperature profile 2 (B, D, and F).

mashes and from 0.1−0.180 U/g to 0.307−0.317 for the BM_{100} mashes. It only decreased slowly upon reaching a mash temperature of 63 °C. The above-mentioned inhibition by wheat was not observed for the β-D-xylosidase activity.

α-L-Arabinofuranosidase Activity. The α-L-arabinofuranosidase activity followed the same curve as that of the endoxylanase activity: it increased from 0.015−0.024 U/g to 0.046−0.054 U/g for the $BM_{60}W_{40}$ mashes and from 0.018−0.024 U/g to 0.071−0.073 U/g for the BM_{100} mashes. The activity also decreased at a mash temperature above 50 °C. Also for the α-L-arabinofuranosidase activity there was no inhibition by wheat.

The stability of the three measured enzymes was apparently not influenced by the addition of wheat. Comparison with the 100% malt mash revealed that the addition of 40% wheat did not influence the thermal stability of the xylanolytic enzymes, but the earlier report (Debyser et al., 1997a) of inhibition of the barley endoxylanase by wheat was confirmed.

β-Glucanase Activity. In two cases, the β-glucanase activity during mashing was also measured (Figure 4). The activities of different endo-acting enzymes active on barley β-glucan were measured, i.e., endo-(1→4)-β-glucanases (cellulases), and endo-(1→3,1→4)-β-glucanases (lichenases). The β-glucanase activity increased initially from 0.40 to 0.53 ΔA_{590}/g for a $BM_{60}W_{40}$ mash and from 0.60 to 1.39 ΔA_{590}/g for a BM_{100} mash. The activity decreased rapidly at 50 °C. This temperature is sufficient to inactivate all the β-glucanase activity. The activity was almost completely destroyed after 15 min at 50 °C. The same thermal stabilities were noted for β-glucanases of malt extracts (Denault et al., 1981) and for two β-glucanases isolated from germinated barley (Woodward and Fincher, 1982). As β-glucan solubilase [apparently with carboxypeptidase and esterase activity (Bamforth et al., 1979; Bamforth, 1981)] is still active above 50 °C (Bamforth and Martin, 1981), the β-glucans solubilized during the last period of mashing are not degraded any further. A similar calculation as above for the inhibition of the malt endoxylanases by wheat resulted in a reduction of 13% in the case of the β-glucanase activity. Therefore, not only endoxylanases (Debyser et al., 1997b), but also the barley malt β-glucanases may be inhibited by wheat.

Xylose Levels. As the arabinose measured originated

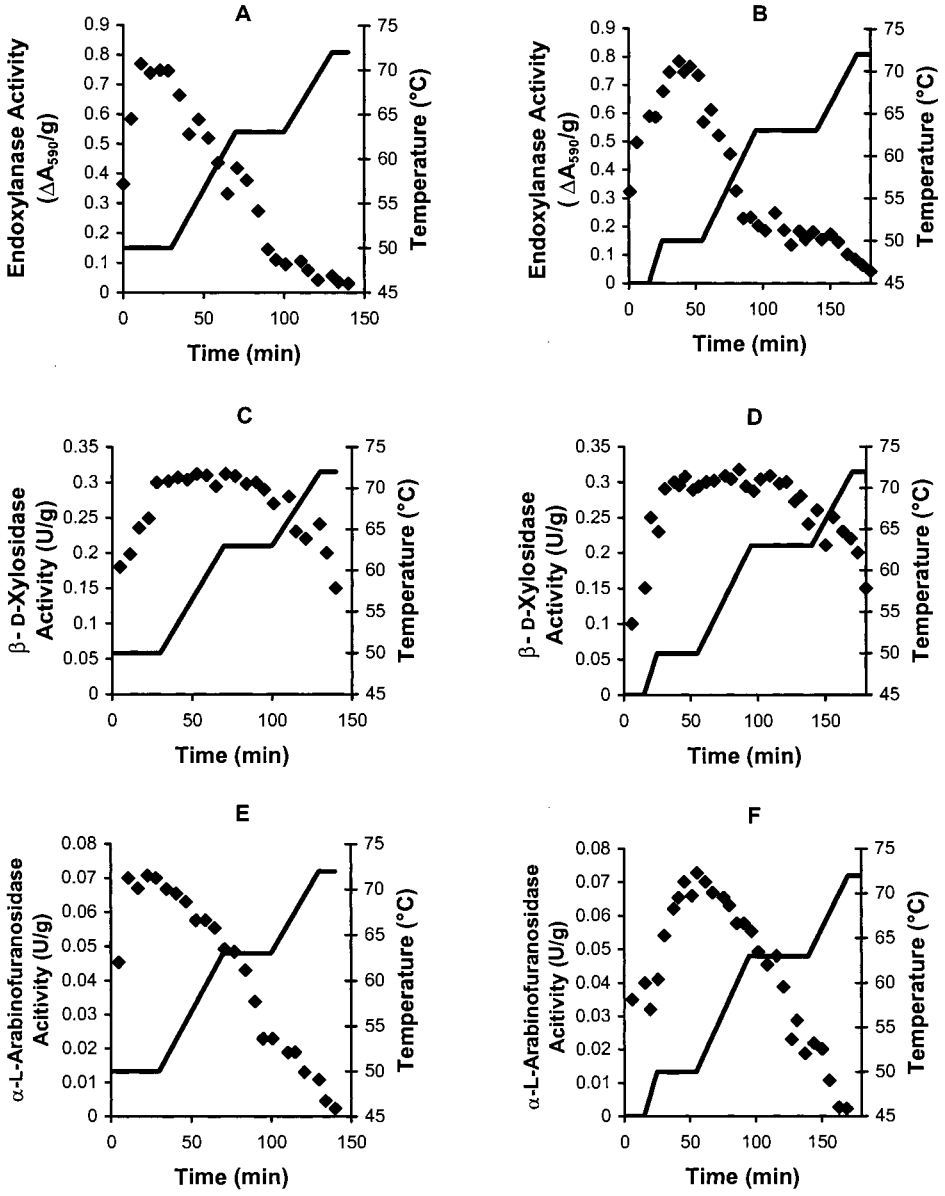

Figure 3. Arabinoxylan hydrolyzing activities (◆) during brewing with 100% barley malt. Typical profiles (—) are shown for temperature profile 1 (A, C, and E) and temperature profile 2 (B, D, and F).

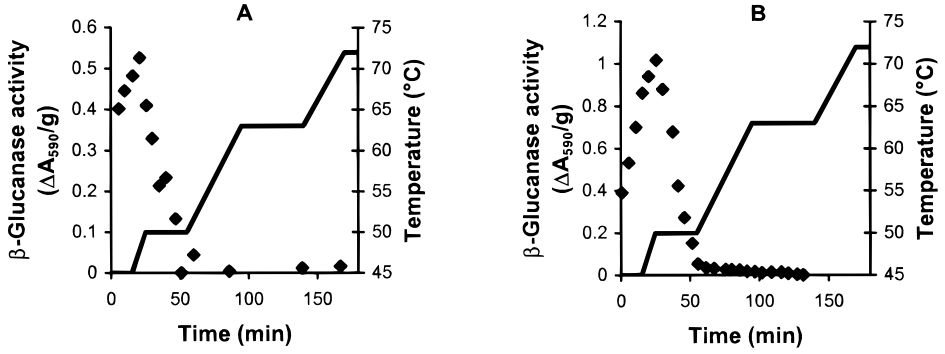

Figure 4. β-Glucanase activities (◆) during mashing with (A) 60% barley malt and 40% wheat and (B) 100% barley malt. Typical profiles (—) are shown for temperature profile 2.

from both arabinoxylan and arabinogalactan, and as up to equal amounts of both can be present in a water extract of wheat (Loosveld et al., 1997), we use the Xyl figures (Table 1) as a relative measure for arabinoxylan levels as done previously (Debyser et al., 1997b). Thus, the increase in levels of xylose-containing material during brewing, is indicative of arabinoxylan solubilization during brewing. The Xyl levels were lower for the $BM_{60}W_{40}$ worts (0.92−1.11 g/L) than for the BM_{100} worts (1.28−1.33) g/L. The hopped $BM_{60}W_{40}$ worts contain 5−10% less arabinoxylan than the corresponding sweet worts. The other steps in beer production

Table 1. Levels of Xylose-Containing Material during Different Steps of Brewing with 60% Barley Malt and 40% Wheat and with 100% Barley Malt[a]

				sweet wort Xyl				
malt	wheat	temp p	WE Xyl (g/100 g)[b]	(g/100 g)[b]	(g/L)	hopped wort Xyl (g/l)	green beer Xyl (g/l)	lautered beer Xyl (g/l)
Plaisant	Soissons	TP1	0.40	0.52	0.92	0.88	n.d.	0.89
Plaisant	Soissons	TP2	0.40	0.54	0.94	0.87	0.85	0.86
Plaisant	Skirlou	TP1	0.50	0.61	1.05	0.99	1.00	0.98
Plaisant	Skirlou	TP2	0.50	0.63	1.11	1.06	1.05	1.07
Plaisant		TP1	0.51	0.74	1.28	n.d.	n.d.	n.d.
Plaisant		TP2	0.51	0.77	1.33	n.d.	n.d.	n.d.
			E.E. < 6%	E.E. < 6%	E.E. < 6%	E.E. < 4%	E.E. < 5%	E.E. < 6%

[a] Abbreviations: temp p, temperature profile; WE Xyl, levels of water-extractable xylose-containing material in starting material; TP1, temperature profile 1; TP2, temperature profile 2; E.E., experimental error. [b] Expressed on dry matter basis of the starting materials.

Table 2. Polymeric β-Glucan Levels during Different Steps of Brewing with 60% Barley Malt and 40% Wheat and with 100% Barley Malt[a]

			polymeric β-glucan		
			sweet wort		
malt	wheat	temp p	(mg/100 g)[b]	(mg/L)	lautered beer (mg/L)
Plaisant	Soissons	TP1	72	128	112
Plaisant	Soissons	TP2	68	117	99
Plaisant	Skirlou	TP1	79	136	118
Plaisant	Skirlou	TP2	69	126	106
Plaisant		TP1	117	192	n.d.
Plaisant		TP2	106	174	n.d.
			E.E. < 8%	E.E. < 8%	E.E. < 8%

[a] Abbreviations: temp p, temperature profile; TP1, temperature profile 1; TP2, temperature profile 2; E.E., experimental error. [b] Expressed on dry matter basis of the starting materials.

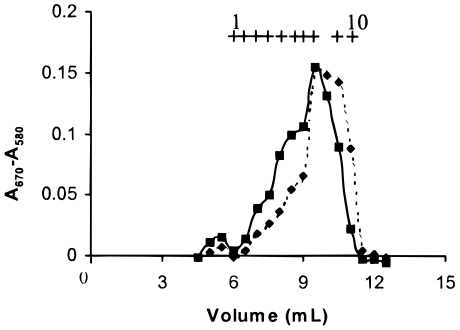

Figure 5. Gel permeation profiles on Shodex B-804 of wort arabinoxylans from 60% barley malt and 40% wheat (■) and 100% barley malt (◆). Retention times of pullulan standards (with molecular weight 78.8×10^4 Da, 40.4×10^4 Da, 21.2×10^4 Da, 11.2×10^4 Da, 3.74×10^4 Da, 2.28×10^4 Da, 1.18×10^4 Da, and 0.59×10^4 Da), xylopentaose (678 Da), and xylose (150 Da) are indicated from 1 through 10, respectively.

apparently do not have an effect on the levels of arabinoxylan. It seems that a part of the arabinoxylans coprecipitated with the proteins and polyphenols during wort boiling. In the literature, a precipitation of up to 3% (Steiner, 1968) is mentioned, but our figures for the $BM_{60}W_{40}$ worts are much higher possibly due to the use of 40% wheat.

As with TP2 the endoxylanases have more time to solubilize arabinoxylans than using TP1, the Xyl levels are somewhat higher for TP2 than for TP1.

Molecular Weight Profiles Polymeric Arabinoxylan. Molecular weight profiles of polymeric sweet wort arabinoxylans are shown in Figure 5. Only one profile of polymeric arabinoxylan in case of BM_{100} and one in case of $BM_{60}W_{40}$ (both with TP2) is shown. The use of 40% wheat seems to increase slightly the molecular weight of the arabinoxylans present in wort. The wheat water-extractable arabinoxylans have much higher molecular weights than what we found in wort (Cleemput et al., 1993).

Polymeric β-Glucan Levels. The polymeric β-glucan levels in worts before boiling and in beer are given in Table 2. Starting the mashing at 45 °C resulted in lower polymeric β-glucan levels. The longer the β-glucanases are active the lower the polymeric β-glucan content. The differences between the two wheat varieties were within the experimental error. The replacement of 40% barley malt by wheat decreased the β-glucan levels of the resulting worts: 174 and 192 mg/L for the BM_{100} worts and 117−136 mg/L for the $BM_{60}W_{40}$ worts.

CONCLUSION

The replacement of 40% barley malt with wheat does not influence the thermal stability of the arabinoxylan degrading enzymes during mashing. During mashing, both endoxylanases and α-L-arabinofuranosidases are stable up to 50 °C and progressively lose activity above this temperature such that at 72 °C they are almost completely destroyed. In contrast, the β-D-xylosidases are still active at the end of the mashing. The inhibition of barley malt endoxylanases during mashing by wheat extractables was confirmed. The β-glucanases, less stable than the arabinoxylan degrading enzymes, were completely inactivated after 15 min at 50 °C. In a classical mashing scheme, the period over which β-glucan degradation occurs, is much shorter than the period over which arabinoxylan is degraded. As a consequence, although barley malt endoxylanases are inhibited by wheat, there is still enough activity to degrade the wheat and barley malt arabinoxylan which can be found in the wort.

ABBREVIATIONS USED

AZCL, azurine cross-linked; BM_{100}, 100% barley malt; $BM_{60}W_{40}$, 60% barley malt and 40% wheat; TP1, tem-

perature profile 1; TP2, temperature profile2; Trizma base, tris[(hydroxymethyl)amino]methane; Xyl, xylose.

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