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Food Chemistry 90 (2005) 101–108
Food Chemistry
www.elsevier.com/locate/foodchem

Control of arabinoxylan solubilization and hydrolysis in mashing

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Received 24 November 2003; received in revised form 16 March 2004; accepted 16 March 2004

Abstract

The effects of mashing variables, such as mashing-in time, mash thickness, grist coarseness, and stirring, on the solubilization and hydrolysis of arabinoxylans were studied. Response surface methodology (RSM) and central composite design (CCD) were employed to further optimize the process. Under the optimum operational conditions, namely mashing-in temperature of 49 °C, first saccharification rest temperature of 62 °C and second saccharification rest temperature of 71 °C, arabinoxylan content decreased from 1510 to 1170 mg/l.

Keywords: Arabinoxylans; Endo-xylanase; Mashing

1. Introduction

Arabinoxylan is the major constituent of walls in the starchy endosperm of barley grain. It can be extracted from cell walls with hot water and can form solutions of high viscosity. This property can be attributed to the asymmetrical molecular conformation. The enzymes that degrade arabinoxylans are often produced late in the germination process (Dennis, 1998), and high levels of this polysaccharide can survive through the brewing process into the final beer (Coote & Kirsop, 1976; Viëtor, Voragen, & Angelino, 1993). So Arabinoxylans from barley grains are responsible for problems in the beer brewing industry, such as low extract yields, high wort viscosity, decreased rate of filtration or haze formation (Viëtor, Voragen, Angelino, & Pilnik, 1991) in beer.

Historically, reduced beer filtration efficiency has been mainly attributed to β-glucan, another important non-starch polysaccharide in barley grain. In fact, it has been reported that the amount of arabinoxylan in commercial beer is ≈10 times (Schwarz & Han, 1995) greater than that of β-glucan. Paul, Paul, and Richard (2002) research indicated that the effects of arabinoxylans on viscosity and filterability are at least as important as the effects of β-glucan. Stewart, Hawthorne, and Evans (1998) found that pilot-brewed beer viscosity and membrane filterability were correlated with arabinoxylan content, whereas β-glucan was correlated only with viscosity. Some arabinoxylans are solubilized from the cell walls but are not extensively degraded by endogenous enzymes during malting. This means that, in malt grist, there are particles containing arabinoxylans, which may be solubilized and hydrolyzed during mashing. This is always the case when unmalted grists are used as adjuncts. But there are few reports in this field.

Solubilized arabinoxylans are, therefore, responsible for high viscosity of malt water-extract, which possibly leads to problems, such as a diminished rate of wort or beer filtration. Therefore, it is important for the brewer to know which parameters affect arabinoxylan solubilization and hydrolysis in mashing. In this paper, the effects of mashing variables, such as temperature programme, mash thickness, coarseness of grists and stirring were studied.

2. Materials and methods

2.1. Materials

In the laboratory-scale mashing, a two-row barley variety Harrington from Canada was used. Finely milled

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doi:10.1016/j.foodchem.2004.03.031

barley, wheat and malted wheat were used as adjuncts. All chemicals were of analytical grade. A kit of neutral sugars and 1-methylimidazole were bought from Sigma Co., Ltd.

2.2. Laboratory scale mashing

Laboratory scale mashing experiments were carried out in a mashing bath. 50 g of ground malt were suspended in 200 ml of prewarmed deionized water containing 75 mg $CaCl_2 \cdot 2H_2O$ and 0.3 ml 0.5 M H_2SO_4. The stability of endo-xylanase and the solubilization of arabinoxylans were studied during seven isothermal mashings at 40, 45, 50, 55, 60, 70, and 80 °C.Samples were taken at intervals of 10–20 min. They were immediately cooled to 4 °C and centrifuged at $4000 \times g$ for 15 min to prevent further enzymatic conversion and sugar extraction. Then, samples were filtered through a micromembrane filter (0.45 μm, Satorious, Germany) before analysis.

Response surface methodology (RSM) was used to study the effects of temperature during mashing-in and saccharification rests on the arabinoxylans in wort, using a 3×3 factorial central composite design (CCD), according to Box and Benhnken (1960) method. Based on preliminary experiments, three independent variables were mashing-in temperature (°C , X_1), first saccharification rest temperature (°C , X_2) and second saccharification rest temperature (°C, X_3), and the dependent variable was arabinoxylan content in wort (mg/l, $Y1$). The length of each rest was 30 min. At the end of the mashings, an additional 10 min at 80 °C for mashing-out was held in all the trials. The effects of temperature rests on arabinoxylan concentration were analyzed using the response surface experiment design of the Statistical Analysis System program (SAS8.1). Regression analysis was performed using the coded values (x_1, x_2 and x_3) of the control temperatures.

The influence of the length of the mashing-in period on the arabinoxylan content in the final wort was studied using a temperature of 49 °C, which is the optimum temperature for arabinoxylan hydrolysis. The length of the rest was varied from 0 to 60 min. On the basis of the results of the optimization trials, the mashing programme shown in Fig. 7 (see below) was chosen for studying the effects of mashing thickness, grist coarseness, and stirring on the arabinoxylan content in final wort.

2.3. Variation of mash thickness

Mash thickness was varied between 1:2.5 and 1:5 (grist to water ratio) to study its effect on content of arabinoxylan in final worts. The extract contents and arabinoxylans contents of the final worts were analyzed.

2.4. Variation of grist coarseness

The fine grist and coarse grist were produced by a pilot mill (mode A, Miag, Braunschweig, Germany). Endo-xylanase activity and arabinoxylan content were analyzed in the samples taken during mashing.

2.5. Variation of stirring

The effects of continuous stirring, intermittent stirring at 10 min intervals and no stirring on arabinoxylan content in wort were studied. The arabinoxylan contents of the wort at each end of the rest saccharification were analyzed.

2.6. Statistical analysis

A Statistical Analysis System (SAS8.1) software package was used for analysis of variance and to fit the second order models to the dependent variables using the following equation:

$$Y_i = A_0 + \sum A_i X_i + \sum A_{ii} X_i^2 + \sum A_{ij} X_i X_j,$$

where A_0, A_i, A_{ii}, A_{ij} are constant and regression coefficients of the model, and X_i the independent variables in coded values. These graphs were drawn by imposing a constant value equal to zero (central point) on one or two of the three independent variables.

2.7. Analytical methods

Endo-xylanase activity in each sample was determined by the DNS method, according to the method of Bailey, Biely, and Poutanen (1992).

Arabinoxylan contents were determined by measuring total monosaccharide composition. Monosaccharide composition of biological samples has been widely determined by acid hydrolysis of the polysaccharides, followed by conversion of the monosaccharides to alditol acetates, and analyzed by gas chromatography. Worts were centrifuged at $3000 \times g$ for 10 min and hydrolyzed for 90 min with 2.0 M trifluoroacetic acid (121 °C) (Debyser, Derdelinckx, & Declour, 1997a). Alditol acetates were prepared based on the method of Englyst and Cummings (1984). Seperation 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. Arabinoxylan contents were calculated as $0.88 \times (\%\ \text{arabinose} + \%\ \text{xylose})$ (Henry, 1986).

3. Results and discussion

3.1. Isothermal mashings

During the mashing period, endo-xylanase in grist dissolved and degraded arabinoxylans into oligo-β-xylosides.

Endo-xylanase was inactivated slowly at 40, 45, 50 °C and very rapidly at temperatures above 55 °C (Fig. 1). The relative endo-xylanase activities at 55 and 60 °C were 58.1% and 27.3%, respectively. There was almost no endo-xylanase activity detected at higher temperatures.

During the first 20 min, there were small amounts of arabinoxylans detected at 40, 45 and 50 °C. This may be explained by solubilized arabinoxylans in grist being hydrolyzed by activated endo-xylanase. It was reported that parts of the insoluble arabinoxylans are converted into soluble arabinoxylans at high temperature (Home, Wilhelmson, & Autio, 1999; Saulnier, Gevaudan, & Thibault, 1994; Suhasini, Muralikrishna, & Malleshi, 1997). When mashing temperature was above 55 °C and mashing time was after 40 min, a rapid rate of arabinoxylan release occurred (Fig. 2). The content of arabinoxylans in final wort at 80 °C was 1638 mg/l and was much higher than it at 40 °C (996 mg/l).

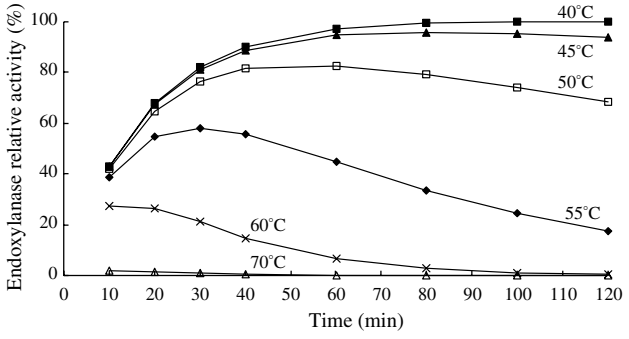

Fig. 1. Endo-xylanase relative activities in isothermal mashings.

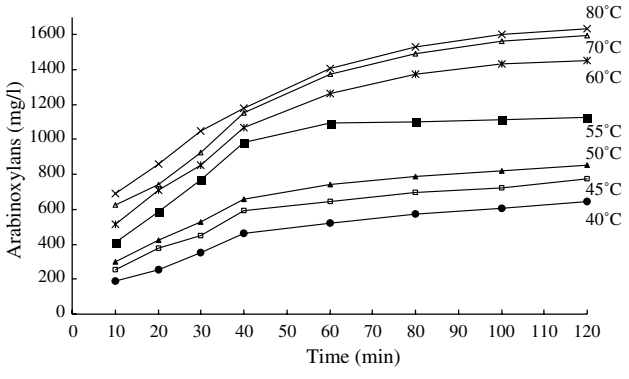

Fig. 2. Arabinoxylan content in isothermal mashings.

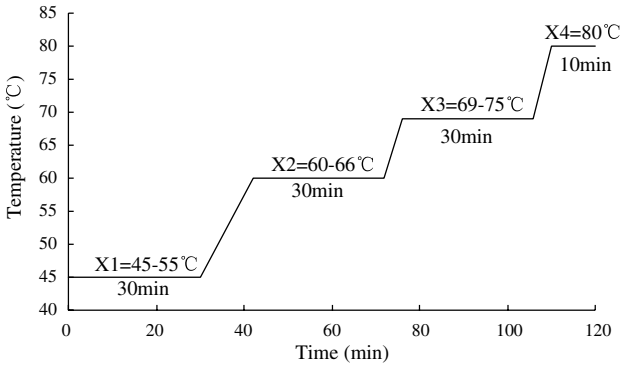

Fig. 3. Variation of the mashing-in temperature and rest temperatures used in the trials for optimization of the mashing programme.

3.2. Optimization of the process employing CCD and RSM strategies

Fifteen mashings were prepared for response surface analysis. The temperature programme consisted of a mashing-in temperature and two saccharification rest temperatures. The results of the regression analysis were presented as response surface.

Central composite design and response surface methodology strategies were adopted (Fig. 3) as an efficient way to find the optimum conditions for arabinoxylan solubilization and hydrolysis in mashing, which included mashing-in temperature (X_1), first saccharification rest temperature (X_2) and second saccharification rest temperature (X_3). Experiments and response values (Table 1) were then used to obtain a full second order polynomial model by a multiple regression (Box & Benhnken, 1960).

The regression models were tested for adequacy by analysis of variance. The close R squared (0.9910) to 1, a large F-value (61.09), and a small value of coefficient variance (1.37), implied that the model was strong and can well predict responses, and 99% of the experimental variation can be explained by it.

Regression coefficients, as well as their student's t-test values, for the models of arabinoxylan solubilization and hydrolysis, are presented in Table 2. Mashing-in temperature and first saccharification rest temperature were the important factors affecting arabinoxylan contents, the optima being 49–50 and 62–63 °C (Figs. 4 and 5), respectively. Mashing-in temperature was the key factor influencing arabinoxylan concentration, due to its largest t-value among the three variables. Second saccharification rest temperature exerted a slighter effect (not significant at 99% level) on the arabinoxylan content, than the other two independent variables. The surface plot and contour plot (Fig. 6) showed that the minimum arabinoxylan content was located near the central point of the mashing-in temperature, indicating that the arabinoxylan content in wort can be partially controlled by adjusting mashing-in temperature.

Table 1
The 3×3 factorial central composite designs and response values for temperature optimization of arabinoxylan solubilization and hydrolysis

Experiment number	Real values			Coded values			$Y1$
	X_1	X_2	X_3	x_1	x_2	x_3	mg/l
1	45	60	72	−1	−1	0	1367
2	45	66	72	−1	1	0	1395
3	55	60	72	1	−1	0	1461
4	55	66	72	1	1	0	1510
5	50	60	69	0	−1	−1	1221
6	50	60	75	0	−1	1	1242
7	50	66	69	0	1	−1	1269
8	50	66	75	0	1	1	1276
9	45	63	69	−1	0	−1	1324
10	55	63	69	1	0	−1	1495
11	45	63	75	−1	0	1	1350
12	55	63	75	1	0	1	1501
13	50	63	72	0	0	0	1204
14	50	63	72	0	0	0	1210
15	50	63	72	0	0	0	1198

Table 2
Regression coefficients of a full second-order polynomial model for temperature optimization of arabinoxylan solubilization and hydrolysis

Term	Coefficients estimated	t value
A_0	1204.000	114.00[a]
A_1	66.375	10.26[a]
A_2	19.875	3.07[a]
A_3	7.50	1.16
A_{11}	197.375	20.73[a]
A_{12}	5.250	0.57
A_{13}	−5.000	−0.55
A_{22}	31.875	3.35[a]
A_{23}	−3.500	−0.38
A_{33}	16.125	1.69

[a] Significant at 99% level.

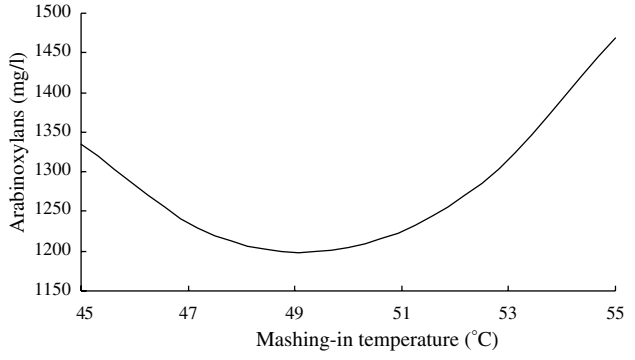

Fig. 4. Effect of mashing-in temperature on arabinoxylan content ($x_2 = 0$, $x_3 = 0$).

Canonical analysis, a mathematical procedure used to simplify a second-order polynomial model, was then employed to investigate the nature of the surface and predict the optimal point. In this model, the stationary point of the current response surface was a minimum

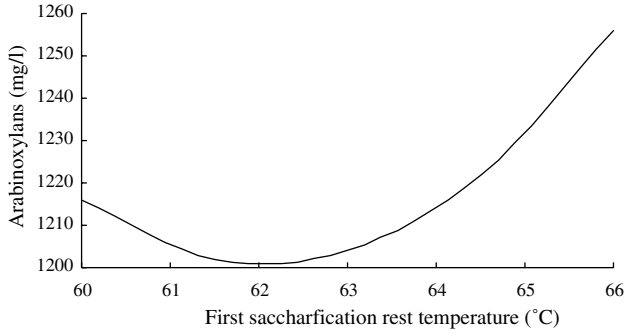

Fig. 5. Effect of first saccharification rest temperature on arabinoxylan content ($x_1 = 0$, $x_3 = 0$).

response. Under the optimum operational conditions, namely mashing-in temperature of 49 °C ,first saccharification rest temperature of 62 °C, second saccharification rest temperature of 71 °C, arabinoxylan content in wort was decreased to 1194 mg/l. Triplicate experiments were carried out to check the fitness of the model. Under the optimal conditions, the mean value of arabinoxylan content in wort was decreased from 1510 (Table 1) to 1170 mg/l. The observed value and predicted response were in close agreement. So the effects of temperature programme on arabinoxylan content can be reliably predicted by the model (Fig. 7).

3.3. Mashing-in time

At optimum mashing-in temperature (49 °C), the effects of mashing-in time (0–60 min) on arabinoxylan solubilization and hydrolysis were studied. The effect of adjuncts was also investigated.

In all cases, a rest of 30 min at 49 °C had a marked effect on arabinoxylan content (Fig. 8). Increasing the time beyond 30 min had no effect when 100% barley

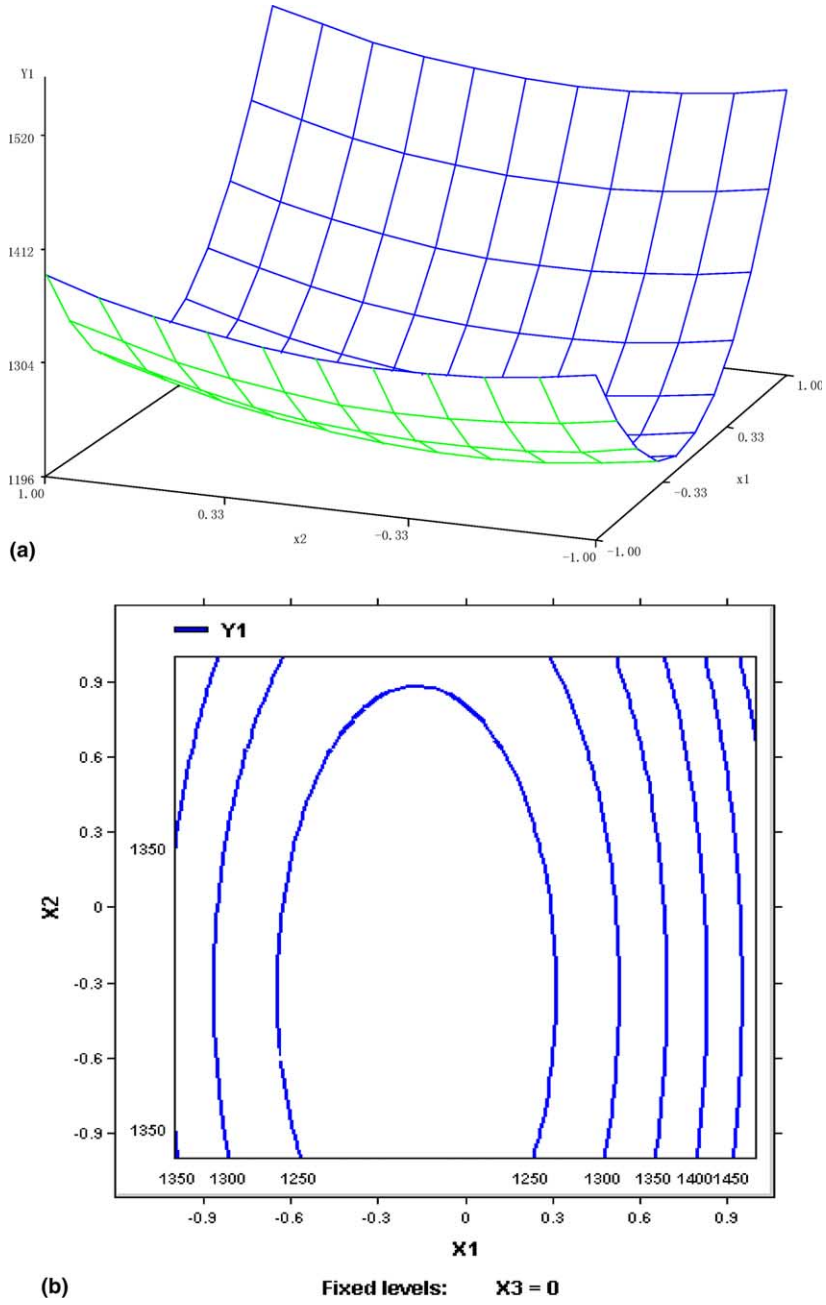

Fig. 6. Effect of mashing-in temperature and first saccharification rest temperature on arabinoxylan solubilization and hydrolysis at second saccharification rest temperature of 72 °C ($x_3 = 0$). $Y1 = 1204 + 66.375 \times x_1 + 19.875 \times x_2 + 197.375x_1 \times x_1 + 31.875x_2 \times x_2$. (a) Surface plot and (b) contour plot.

malt was used and arabinoxylan content was only slightly decreased when adjuncts were used. When finely milled, unmalted barley (at 20% level) was used as an adjunct, the arabinoxylan content in final wort decreased to a level comparable with that of 100% malt wort after a mashing-in time of 60 min. This can be explained in that the malt used supplied sufficient endo-xylanase for hydrolyzing arabinoxylans in barley grist. However, the hydrolysis of arabinoxylans was not intense especially when wheat and malted wheat were used for brewing, since wheat endosperm contains few more arabinoxylans than barley endosperm (Henry, 1985). Another important reason was that the presence of one or more endo-xylanase inhibitors in wheat extract inactivated endo-xylanase activity in barley malt (Debyser, Derdelinckx, & Declour, 1997b; Rouau & Surget, 1998). From mashing-in time 0–60 min, arabinoxylan contents of the wort with wheat and malted wheat decreased by 28.3% and 24.8%, respectively, compared with wort with barley which decreased by 39.2%.

The arabinose-to-xylose (A/X) ratio shows the degree of substitution of the xylan backbone by arabinose

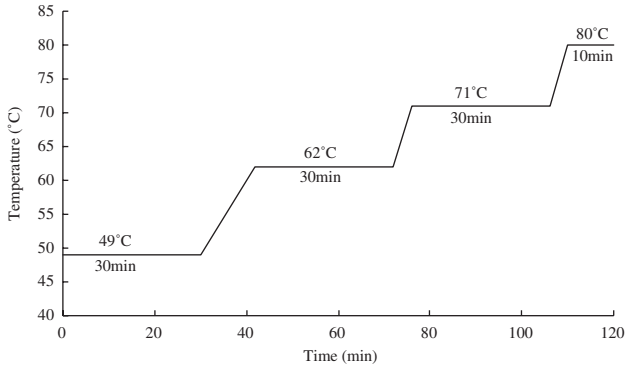

Fig. 7. Temperature programme used to study the effects of mash thickness, grist coarseness, and stirring.

residues. In general, arabinoxylans isolated from cell walls contain more arabinose than those from the husk or aleurone. The A/X ratio of 100% malt wort was 0.69 and a similar value (0.70) was found when unmalted barley (at 20% level) was used as an adjunct. However, when wheat and malted wheat were used as adjuncts, the A/X ratios of their worts were decreased to 0.54 and 0.57, respectively. This may be because the ratio of A/X in arabinoxylans from wheat endosperm may vary from 0.50 to 0.71 (Cleemput, Roels, Vanoort, Grobet, & Delcour, 1993) but it is usually below that found in barley endosperm (0.72). In general, water-extractable barley arabinoxylans were very highly substituted. Viëtor et al. (1993) also speculated that these differences in xylose substitution might be related to the differences in water-extractability. So it is possible that the water-extractables from barley malt are more resistant to endoxylanase degradation, due to a higher proportion of disubstituted xylose residues (Han, 2000).

3.4. Mash thickness

Table 3 shows the effects of grist:water ratio on the solubilization and hydrolysis of arabinoxylans. 100% Barley malt and barley malt with two adjuncts were compared when grist:water ratio varied from 1:2.5 to 1:5. When more diluted mashes were used, more efficient solubilization of arabinoxylans was observed. When 100% barley malt was used, the proportion of arabinoxylans in the extract ranged from 0.679% to 0.710%. The effect was more pronounced with barley and wheat as adjuncts. Because a certain amount of water is bound by starch, the water phase in thick mashes becomes concentrated, limiting the solubilization of arabinoxylans.

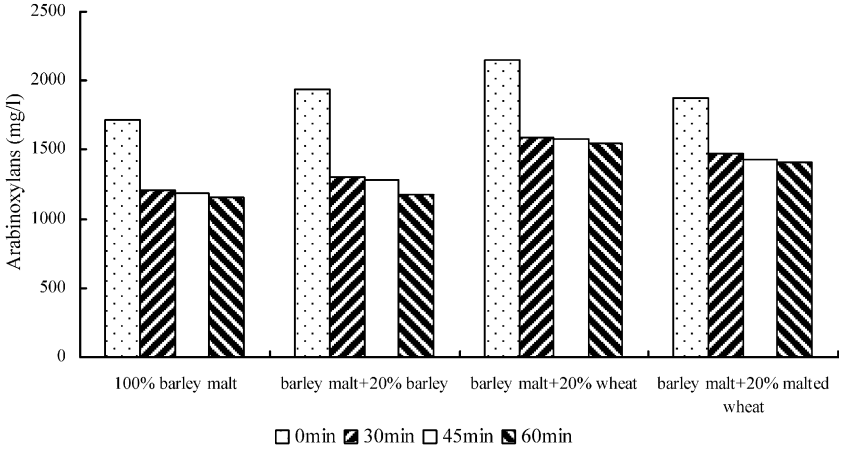

Fig. 8. Effect of mashing-in time on arabinoxylan content in final wort.

Table 3
Effect of grist:water ratio on the solubilization and hydrolysis of arabinoxylans

Grist–water ratio	100% barley malt			80% barley malt + 20% barley			80% barley malt + 20% wheat		
	Arabinoxylans (mg/l)	Extract (g/100 ml)	Arabinoxylans (% of extract)	Arabinoxylans (mg/l)	Extract (g/100 ml)	Arabinoxylans (% of extract)	Arabinoxylans (mg/l)	Extract (g/100 ml)	Arabinoxylans (% of extract)
1:2.5	1760	25.9	0.679	1812	24.5	0.739	2060	25.1	0.821
1:3.0	1490	21.8	0.683	1605	21.5	0.747	1866	21.6	0.864
1:3.5	1322	19.2	0.689	1410	18.1	0.779	1623	18.5	0.877
1:4.0	1180	16.9	0.698	1285	16.2	0.793	1443	16.4	0.879
1:4.5	1078	15.4	0.700	1181	14.7	0.803	1388	15.1	0.919
1:5.0	966	13.6	0.710	1080	13.2	0.818	1249	13.2	0.946

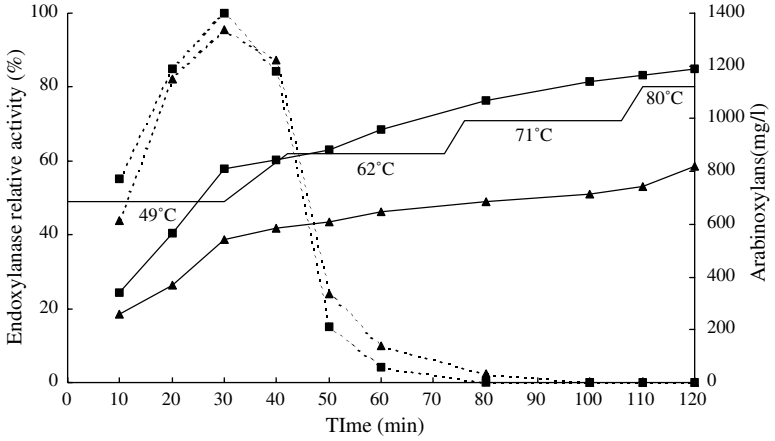

Fig. 9. Effect of grist coarseness on endo-xylanase activities (- - - - -) and arabinoxylan concentrations (——) during mashing. Fine grist (■), coarse grist (▲).

3.5. Coarseness of grists

The effect of grist coarseness on arabinoxylan solubilization and hydrolysis was studied using a fine grist and a coarse grist (Hermia & Rahier, 1992). As shown in Fig. 9, there was no significant difference in enzyme activities between the grist types. After mashing for 30 min, both enzyme activities reached maxima. But the solubilization and hydrolysis of arabinoxylans in two grist types differed greatly. Results (Fig. 9) suggest that arabinoxylan content of fine grist was consistently greater than that of coarse grist. The arabinoxylan contents in final wort of fine grist and coarse grist were 1187 and 820 mg/l, respectively. The coarse grist probably limited the solubilization, resulting in low arabinoxylans concentrations in the wort.

3.6. Stirring method

The effect of stirring on arabinoxylan solubilization and hydrolysis was studied using continuous stirring, stirring at 10 min intervals and no stirring. Table 4 shows that the stirring method affected the solubilization of arabinoxylans. When the stirring was more effective, more arabinoxylans were released. The arabinoxylan content in the continuously stirred mash was almost double that in the un-stirred mash. It seems that physical and mechanical forces might have a marked effect on the solubilization of arabinoxylans.

Table 4
Effect of stirring on the solubilization and hydrolysis of arabinoxylans

Test point	Arabinoxylans (mg/l)		
	Continuous stirring	Intermittent stirring	No stirring
I[a]	607	493	380
II[a]	939	719	515
III[a]	1142	879	616
IV[a]	1190	975	786

[a] Determination were performed at the end of the 30 min mashing-in time at 49 °C (I), at the end of the 30 min rest at 62 °C (II), at the end of the 30 min rest at 71 °C (III), and at the end of the 10 min rest at 80 °C (IV).

4. Conclusions

The solubilization and hydrolysis of arabinoxylans during mashing were investigated. During isothermal mashing periods, there was almost no endo-xylanase activity detected at temperatures above 70 °C and the arabinoxylan content at 80 °C was much greater than that at 40 °C. CCD and RSM were employed to further optimize the process. After the optimization, the content of arabinoxylans in final wort was down to 1170 mg/l. By increasing the mashing-in time, arabinoxylans in 100% barley malt wort, and wort with unmalted barley as an adjunct, effectively decreased to a low level. But the hydrolysis of arabinoxylans in wort with wheat and malted wheat was not intense due the presence of endo-xylanase inhibitors in wheat extracts.

Mash thickness also affected the solubilization and hydrolysis of arabinoxylans. More efficient solubilization of arabinoxylans was observed with increase of the grist:water ratio. The effect was more pronounced when unmalted adjuncts were used. With the optimal mashing programme used, fine milling resulted in higher arabinoxylan content in the wort. And agitated stirring would accelerate the solubilization of arabinoxylans.

In this work, the focus was on ways to minimize the arabinoxylan content in wort. The effects of mashing variables on the solubilization and hydrolysis of arabinoxylans were studied. Our further researches on establishing a model of dissolution and denaturation of endo-xylanase and solubilization and hydrolysis of arabinoxylans are now in progress. Through the model,

the effects of changes in the mashing-in variables and malt properties on arabinoxylan concentration can be predicted on the laboratory scale and industrial scale, to avoid high arabinoxylan content in wort.

References

Bailey, M. J., Biely, P., & Poutanen, K. (1992). Interlaboratory testing of methods for assay of xylanase activity. *Journal of Biotechnology, 23*, 257–270.

Box, G. E. P., & Benhnken, D. W. (1960). Some new three level design for the study of quantitative variables. *Technometrics, 2*, 456–475.

Cleemput, G., Roels, S. P., Vanoort, M., Grobet, P. J., & Delcour, J. A. (1993). Heterogeneity in the structure of water-soluble arabinoxylans in European wheat flours of variable bread-making quality. *Cereal Chemistry, 70*, 324–329.

Coote, N., & Kirsop, B. H. (1976). A haze consisting largely of pentosan. *Journal of Institute Brewing, 82*, 34.

Debyser, W., Derdelinckx, G., & Declour, J. A. (1997a). Arabinoxylan and arabinoxylan hydrolysing activities in barley malts and worts derived from them. *Journal of Cereal Science, 26*, 67–74.

Debyser, W., Derdelinckx, G., & Declour, J. A. (1997b). Arabinoxylan solubilization and inhibition of the barley malt xylanolytic system by wheat during mashing with wheat wholemeal adjunct: evidence for a new class of enzyme inhibitors in wheat. *Journal of the American Society of Brewing Chemists, 55*, 153–156.

Dennis, E. B. (1998). *Malts and malting*. London: Blackie Academic and Professional.

Englyst, H. N., & Cummings, J. H. (1984). Simplified method for the determination of total non-starch polysaccharides by gas-liquid chromatography of constituent sugars as alditol acetates. *Analyst, 109*, 937–942.

Han, J. Y. (2000). Structural characteristics of arabinoxylan in barley, malt, and beer. *Food Chemistry, 70*, 131–138.

Henry, R. J. (1985). Comparison of the non-starch carbohydrates in cereal grains. *Journal of the Science of Food and Agriculture, 36*, 1243–1253.

Henry, R. J. (1986). Genetic and environmental variation in the pentosan and β-glucan contents of barley and their relation to malting quality. *Journal of Cereal Science, 4*, 269–277.

Hermia, J., & Rahier, G. (1992). The 2001 filter: a new technology for sweet wort production. *Ferment, 5*, 280–286.

Home, S., Stenholm K., Wilhelmson, A., & Autio, K. (1999). Properties of starch and cell wall components and their effects on processing. In *Proceedings of the nineth australian barley technical symposium*.

Paul, S., Paul, B. S., & Richard, D. H. (2002). Effect of arabinoxylans, β-glucans, and dextrins on the viscosity and membrane filterability of a beer model solution. *Journal of the American Society of Brewing Chemists, 60*, 153–162.

Rouau, X., & Surget, A. (1998). Evidence for the presence of a pentosanase inhibitor in wheat flours. *Journal of Cereal Science, 28*, 63–67.

Saulnier, L., Gevaudan, S., & Thibault, J.-F. (1994). Extraction and partial characterization of β-glucan from the endosperms of two barley cultivars. *Journal of Cereal Science, 19*, 171–178.

Schwarz, P. B., & Han, J. Y. (1995). Arabinoxylan content of commercial beers. *Journal of the American Society of Brewing Chemists, 53*, 157–159.

Stewart, D. C., Hawthorne, D., & Evans, D. E. (1998). Cold sterile filtration: A small scale filtration test and investigation of membrane plugging. *Journal of Institute Brewing, 104*, 321–326.

Suhasini, A. W., Muralikrishna, G., & Malleshi, N. G. (1997). Free sugars and non-starch polysaccharide contents of good and poor malting varieties of wheat and their malts. *Food Chemistry, 60*, 537–540.

Viëtor, R. J., Voragen, A. G. J., Angelino, S. A. G. F., & Pilnik, W. (1991). Non-starch polysaccharides in barley and malt: A mass balance of flour fractionation. *Journal of Cereal Science, 14*, 73–83.

Viëtor, R. J., Voragen, A. G. J., & Angelino, S. A. G. F. (1993). Composition of non-starch polysaccharides in wort and spent grain from brewing trials with malt from a good malting quality barley and a feed barley. *Journal of Institute Brewing, 99*, 243–248.