PUBLICATION I

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Impact of water content on the solubilisation of arabinoxylan during xylanase treatment of wheat bran

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A B S T R A C T

Arabinoxylan (AX) has a major impact on the functional properties of wheat bran, and it has been shown that technological properties of bran can be improved by using endoxylanases. Enzymatic treatments are typically conducted at high water content. However, in industrial applications, low water content may be advantageous, especially when targeting dry end products. The aim of the study was to examine the impact of water content, ranging from 20 to 90%, on the efficiency of endoxylanase treatment of wheat bran. Interestingly, AX solubilisation was highest at the water contents of 40 and 90%. At water contents 50–80%, AX solubilisation was lower than at 40 and 90%. Furthermore, at low water content, less depolymerisation was detected. At water content of 40%, the bran-water mixture was transformed from powder-like into compact mass. Probably the compact consistency of the material enhanced AX solubilisation by increased breakdown of bran cell walls due to shear forces or via enhanced enzyme binding to the substrate. The results show that solubilisation of bran AX can also be efficiently performed at low water content.

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1. Introduction

Bran is an important by-product of the cereal industry, and comprises the outer tissues of the grain. Bran is rich in dietary fibre (DF), and is composed mainly of cell wall material. Different cell wall components, such as cellulose, arabinoxylans (AX), beta-glucans, proteins and phenolic compounds, are associated with each other both non-covalently and covalently, constituting a complex network (Iiyama et al., 1994).

Several studies have shown that whole grain consumption and intake of DF reduce the risk of chronic diseases (Mellen et al., 2008, de Munter et al., 2007). Many of these beneficial effects have been ascribed to cell wall polysaccharides such as AX and beta-glucans. The use of whole grain or bran in food applications, however, is limited due to challenges in technological and sensory properties of the bran fraction. The complex nature of the cell wall also partly restricts the bioavailability of potentially health promoting compounds, such as phenolic compounds (Vitaglione et al., 2008).

The technological and nutritional functionality of bran can be improved by enzymatically modifying the complex insoluble network structure of the cell walls (Anson et al., 2009; Katina et al., 2006). Endoxylanases (EC 3.2.1.8) are hydrolytic enzymes that cleave the backbone of AX, decreasing the degree of polymerisation, increasing solubility of the polymer and releasing xylo-oligosaccharides of varying composition. In cereal processing, xylanases are increasingly used; e.g. in the baking industry because of their ability to improve textural properties of bread (Courtin and Delcour, 2002). Cereal materials also contain endogenous xylanolytic activity, which plays an important role for example in fermentation processes (Katina et al., 2007). Xylanase specificity, substrate selectivity and synergistic action with other enzymes have been studied using varying cereal AX fractions and bran components as substrate (Beaugrand et al., 2004; Benamrouche et al., 2002; Courtin and Delcour, 2001; Faulds et al., 2006; Moers et al., 2005). Xylanases have been applied for example for solubilisation of rye AX (Figueroa-Espinoza et al., 2004), increasing the amount of soluble DF (Napolitano et al., 2006) and for production of oligosaccharides (Swennen et al., 2006; Yuan et al., 2006).

In the majority of the studies, the enzyme reactions have been conducted in highly aqueous systems, typically over 90% water content. However, high water content may not be feasible in industrial scale applications, especially if the product will be dried after the enzymatic treatment. On the other hand, water content also affects the mass transfer and rheological properties of the

Abbreviations: AX, arabinoxylan; DF, dietary fibre; FU, farinograph resistance unit; dm, dry matter; HP-SEC, high performance size exclusion chromatography; Mw, molecular weight; WEP, water extractable pentosans; DS, degree of solubilisation.

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reaction mixture, which may pose technological challenges at lower water contents.

Enzymes need water for their catalytic activity. Water affects enzyme reactions and stability in various ways, influencing enzyme structure via non-covalent bonding and disruption of hydrogen bonds, facilitating reagent diffusion, and influencing the reaction equilibrium (Hari Krishna, 2002). Too low water content generally reduces enzyme activity, but the minimum hydration level for activity varies a lot. Some enzymes can function even in solvent-free reaction conditions on solid substrates, expressing extraordinary reaction patterns (Hobbs and Thomas, 2007). Despite the abundant research in the area of enzymatic modification of bran and AX, there are only a few publications dealing with the effect of water content on the efficiency of these treatments (Napolitano et al., 2006; Sørensen et al., 2006).

The objective of this study was to demonstrate the influence of water content of bran-water mixtures on the solubilisation of AX during xylanase treatment of wheat bran. The relationship between the water content and the physical state (rheology, water activity) of bran-water mixtures in relation to the AX solubilisation and remaining extractable xylanase activity was also evaluated.

2. Experimental

2.1. Materials

The bran was obtained from mixed wheat varieties (Mühle Rüningen GmbH & Co. KG, Braunschweig, Germany). Before bran removal, the grains were peeled to remove 2–3% of the grain outer layers in order to reduce the level of contaminating microbes and enzymes on the surface layers of the grains. The chemical composition was (% of dm): DF 49.5 (including pentosans 21.7, fructan 3.6, beta-glucan 2.8), protein 19.5, starch 11.6, fat 4.8, ash 6.7. The bran was ground in batches of about 4 kg by passing each batch three times through a mill (Hosokawa Alpine, 100 UPZ, Retsch GmbH, Germany; mill sieve size 0.3 mm). After grinding, the mean particle size was about 100 μm, and 90% of the particles were smaller than 390 μm as determined by Coulter Particle size analyser dry module (Coulter Corporation, USA).

A commercial *Bacillus subtilis* xylanase preparation, Depol 761P (Biocatalysts, UK), was used for bran treatments. According to the manufacturer, it is especially suitable for extraction of soluble fibre from wheat bran. The xylanase activity of Depol 761P was 137 000 EU/g, as analysed by the by Xylazyme AX Tablet assay as described in section 2.3.4.

2.2. Enzymatic treatment of wheat bran

Bran was treated with or without added enzyme according to the scheme shown in Fig. 1A. The enzyme preparation was dosed according to its xylanase activity at levels of 97 or 970 EU/g bran. The enzyme powder was mixed with bran before water addition. The water content of a treatment is expressed as the total water content, i.e. the water content of bran was taken in account. The treatments at ≤70% water contents were performed in a farinograph mixing bowl (Brabender Farinograph, mixer type S300 with z-blades, Fig. 1B). The mixer bowl was heated by water circulation (50 °C) and the mixing speed was 60–63 rpm. 125 g of ground bran (with or without enzyme addition) was placed in the mixer, and pre-heated water was added by spraying during 1–3 min while the blades were rotating to obtain an even distribution of water. The mixing bowl was sealed tightly to avoid evaporation of moisture. At higher water contents (80 and 90%), due to the liquid form of the mixture, the reactions were performed in a covered steel container (0.5 l) with double-blade mixer (speed 160 rpm) placed in a water bath (50 °C).

The experiments were done in duplicate. The reaction time varied from 1 to 24 h, after which a sample was taken and reaction stopped by cooling down and freezing the sample immediately. Each sample was divided in two pieces: one part of the sample was used as such for water activity measurement, and the rest of the sample was freeze dried for other analyses. Before analyses, the freeze dried samples were ground with a laboratory mill (0.5 mm sieve). The remaining moisture contents of freeze dried samples were determined by oven drying (1 h at 130 °C).

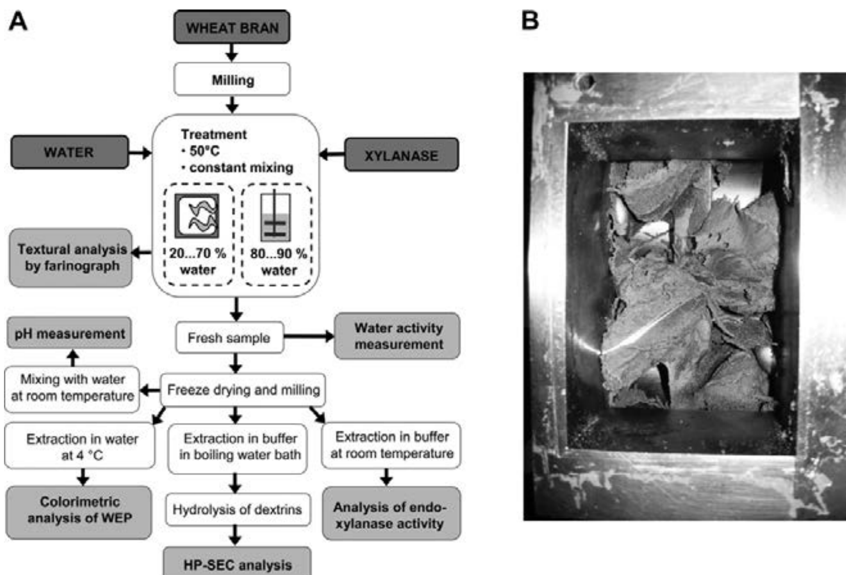

Fig. 1. A) Process scheme for the enzymatic treatments and analyses. B) Bran mixture at water content of 40% in a farinograph mixer after 24 h incubation with xylanase (97 EU/g).

2.3. Analysis methods

2.3.1. Chemical analyses

Analyses of the bran were made as follows: protein content by Kjeldahl method (AACC, 2003b, no. 46–11A), total DF by enzymatic-gravimetric method (AOAC, 1990 method no. 985.29), beta-glucan by AACC (2003a) method no. 32–23, fructan by AOAC (2003) method no. 999.03, fat by AOAC (2000) method no. 922.06, digestible starch by Megazyme method (McCleary et al., 1994) and ash by burning at 550 °C. For the quantification of water extractable pentosan (WEP), 0.25 g of the freeze dried sample was mixed with 8 ml of 4 °C distilled water and shaken with glass pearls for 15 min at 4 °C. After centrifugation, the supernatant was boiled for 10 min in order to inactivate the residual enzyme activity, and centrifuged again. The amount of pentose sugars in the water extracts was determined by a colourimetric phloroclucinol method (Douglas, 1981) using xylose as a standard. For the quantification of total pentosan, a dry sample of 0.1 g was mixed with 5 ml of 0.5 M H_2SO_4 and boiled for 20 min and centrifuged, followed by the colourimetric determination (Douglas, 1981).

2.3.2. Water holding capacity, water activity, consistency and pH

The approximate water holding capacity (approx. WHC) of bran was analysed by a method modified from Quinn and Paton (1979). 0.5 g bran was weighed in a centrifuge tube and the tube with the contents was tared. An adequate amount of water was added and mixed by vortex. The bran suspension was allowed to swell for 60 min at room temperature and centrifuged. The supernatant was discarded and the tube was weighed. The weight difference per gram of bran dm was taken as the approx. WHC. Changes in textural properties during the first 60 min of bran treatments were followed as farinograph torque values reflecting the resistance of the bran-water mixture against the mixing blades. The water activity at the end of 24 h bran treatment was determined from a fresh sample using AquaLab CX2 (Decagon Devices Inc., USA). The pH of treated bran was measured by solubilising 0.2 g of the freeze dried sample in 10 ml of distilled water and stirring for 15 min prior to measurement.

2.3.3. HP-SEC analysis

The effect of bran treatments on the molecular weight (Mw) distribution of water extractable compounds was analysed by high performance size exclusion chromatography (HP-SEC). 0.2 g of freeze dried sample was mixed in 5 ml of 50 mM Na-phosphate buffer (pH 4.7), boiled for 30 min and centrifuged. Starch and beta-glucans were hydrolysed by adding 50 μl of 1:25 or 1:50 diluted Optidex L-400 (Genencor International) saccharifying enzyme solution to 1000 μl of supernatant and incubating the samples with mixing at 60 °C for 4 h. After incubation, the samples were boiled for 30 min and cooled down. Optidex has been pre-tested with commercial rye and wheat arabinoxylans to confirm that under the conditions applied, it affects neither AX molecular weight nor concentration. The liquid chromatograph with Alliance 2690 separation module and M-2414 refractive index detector, consisted of three columns (7.8 × 300 mm) μHydrogel 500, μHydrogel 250 and μHydrogel 120. All the equipment was purchased from Waters Inc. (Milford, MA, USA). The eluent was 0.2% H_3PO_4 at a flow-rate of 0.5 ml/min. The columns were at 60 °C and the injection volume was 100 μl. Pullulan standards (Waters Inc., Milford, MA, USA) ranging from 788 000–5900 Da, maltoheptaose and maltopentose were used for calibration.

2.3.4. Endoxylanase activity assay

The endoxylanase activity was analysed by Xylazyme AX Tablet assay (Megazyme, Ireland). For the extraction of enzymes, 0.15 g of dry sample was mixed with 10 ml of phosphate buffer (25 mM, pH 6.0) and shaken during 60 min at room temperature. After centrifugation, 0.5 ml of suitably diluted supernatant was equilibrated in 50 °C water bath for 5 min before the addition of an AX substrate tablet. After 60 min, the reaction was terminated by adding 5 ml of Trizma base solution (2% w/v) and mixed. After 6 min at room temperature, the slurry was mixed again and filtered (Whatman No.1). The absorbance of the filtrate at 590 nm was measured against a substrate/enzyme blank prepared according to the assay instructions. Activity levels were expressed in endoxylanase units (EU) per gram. One EU is the amount of endoxylanase needed to yield an absorbance (at 590 nm) of 1.0 per 60 min of incubation, under the conditions of the assay. For the calculation of the percentage of remaining endoxylanase activity in the enzyme treated bran samples, the initial activity was calculated as the sum of the activity of the added xylanase plus the endogenous activity analysed for of the bran.

3. Results

3.1. Consistency and properties of bran mixtures at different water contents

The approx. WHC of bran was 2.8 g water/g bran dm. At water contents of 20 and 30%, the bran was in the form of powder, and the water activity at the end of 24 h incubation was 0.83 and 0.89, respectively (Table 1). At the water content of 40%, the bran-water mixture was transformed into a very compact, plastic-like mass (Fig. 1B). Increasing water content from 40 to 70% increased the water activity from 0.93 to 0.98, while the appearance of the reaction mixture was like a paste. At the highest water contents, 80 and 90%, the water activity was ≥0.98, and the bran-water mixture was a slurry-type dispersion. Addition of xylanase did not affect the water activity or the appearance of the mixture.

The rheological properties of bran-water mixtures, measured as farinograph resistance units (FU), varied with water content (Table 2). Without added enzyme, the resistance value increased with increasing water content up to the water content of 50%, and then at higher water contents, the resistance value again decreased. Addition of exogenous xylanase increased the resistance values, especially at the water content of 40%.

The initial pH of the bran-water mixture was 6.8. During the first 16 h of incubation, only minor pH changes were noticed, and the pH remained between 6.2 and 6.8 regardless of water content (Table 3). After 24 h, however, at the highest water content, 90%, pH decreased to 4.7.

Table 1
Water activity (measured at 24–27 °C) and appearance of bran mixtures at different water contents. Water activity and appearance were not affected by the addition of xylanase. The water activities are expressed as means of four analysis results (duplicate measurements for each bran sample). The standard deviations were less than 1% of the mean.

Water content (%)	Water activity (after 24 h treatment)	Appearance (after 24 h treatment)
20	0.83	Powder
30	0.89	Powder
40	0.93	Paste
50	0.96	Paste
60	0.97	Paste
70	0.98	Paste
80	≥0.98[a]	Slurry
90	>0.98[a]	Liquid

[a] With the used measurement system it was not possible to determine the accurate water activity of samples with high amount of free water.

Table 2
The resistance values of bran mixtures after 1 h incubation. The values are approximate values obtained graphically from farinograph curves.

Water content of bran mixture (%)	Resistance value at 60 min (FU)		
	No added enzyme	With xylanase 97 EU/g	With xylanase 970 EU/g
20	60	60	–[a]
30	110	150	200
40	240–270[b]	330–400[b]	400–550[b]
50	280–310[b]	310–340[b]	310–340[b]
60	140	130	–[a]
70	40	40	–[a]

[a] Not determined.
[b] The range of the values refers to the fluctuation of the resistance curve.

3.2. Remaining endoxylanase activity of enzyme treated bran

The total initial endoxylanase activity of the treatment was 982 EU/g bran, which was calculated by summing the endogenous xylanase activity of the bran, 12 EU/g, and the added xylanase activity dosage, 970 EU/g. The recovery of xylanase activity in water extracts of enzyme treated bran samples varied with the treatment time and water content (Table 3). After 24 h treatment at the water content of 40%, with the initial xylanase dosage of 970 EU/g, only 10% of the initial activity could be detected in the bran extract. The recovery was higher both at lower and higher water contents, and the highest recovery after 24 h treatment, 64%, was detected in bran treated at 90% water content.

3.3. Effect of water content and xylanase treatment on the content of water extractable pentosans

The effect of water content and use of xylanase on bran was examined by analysing the content of WEP after 24 h treatment (Fig. 2A). Without addition of exogenous xylanase, at the lowest water content studied, 20%, no significant increase in WEP content was noticed. However, already at the water content of 30%, remarkable increase in WEP content could be noticed. The highest degree of solubilisation (DS, calculated by dividing the WEP content of the sample by the total pentosan content of the bran, which was 21.7% of dm) was monitored at 40% water content (3.8% WEP of total dry matter, corresponding to DS of 18%). When the water content was increased further, the WEP content was again reduced. With added xylanase (dosage 97 EU/g), WEP content reached the highest value both at 90 and 40% water contents (6.9% WEP of total dm at both water contents, DS 32%), and between water contents of 40 and 90%, the WEP content was lowest at 60% water content (5.5% WEP, DS 25%).

However, the impact of added xylanase on the increase in WEP content in the enzyme treatments varied with water content. The contribution of added xylanase was calculated as a percentage (of the difference between the WEP content of control and enzyme treatments) from the WEP content of the enzyme treated sample. At the water content of 90% with added xylanase, 63% of the increase in WEP content was caused by added enzyme, while at the water content of 40%, that was 45%. At the water content of 30, 50, 60, 70 an 80%, with added xylanase, the contribution of added xylanase on the increase in WEP content was 35, 50, 58, 64 and 63%, respectively.

Effect of treatment water content and xylanase addition was also studied as a function of incubation time and xylanase dosage (Fig. 2B). Without added enzyme, the content of WEP was very low after 1 h treatment at all water contents studied (30, 40, 50 and 90%). After 4 h, water content had an impact on the rate in which the WEP content increased, as observed from the (estimated) slopes of the curves in Fig. 2B, and the rate was highest at the water content of 40% and lowest at the water contents of 90 and 30%. With the xylanase dosage of 97 EU/g, increase in the WEP content during the first hour was fastest at the water content of 90%, followed by the water content of 50% (Fig. 2B). This was also the case when higher enzyme dosage (970 EU/g) was used. However, between 4 and 24 h, at the water contents of 50 and 90%, the rates in which the WEP content increased were approximately the same regardless of enzyme dosage, whereas at the lower water contents (30 and 40%), the rates were relatively more increased with higher enzyme dosage. After 24 h treatments with the enzyme dosage of 970 EU/g, WEP content was highest at the water content of 40% (11.8% WEP, DS 54%), followed by the water content of 90% (10.6% WEP, DS 49%) and 50% (9.5% WEP, DS 44%).

Table 3
pH of bran samples and remaining endoxylanase activity of water extracts of bran samples treated at 30, 40, 50 and 90% water contents with xylanase (970 EU/g). The results are expressed as means of four analysis results (duplicate analyses for each bran sample). The standard deviations were less than 5% of the mean in pH results. The standard deviations of the endoxylanase activity results are indicated in parentheses.

Water content (%)	pH				Endoxylanase activity (% initial)			
	1 h	4 h	16 h	24 h	1 h	4 h	16 h	24 h
30	6.8	6.7	–[a]	6.4	77 (±5)	55 (±2)	–[a]	33 (±5)
40	6.6	6.4	6.3	6.3	81 (±3)	45 (±3)	13 (±2)	10 (±1)
50	6.6	6.4	–[a]	6.3	85 (±1)	67 (±2)	–[a]	63 (±4)
90	6.5	6.5	6.2	4.7	80 (±3)	70 (±2)	66 (±2)	64 (±4)

[a] Not determined.

3.4. HP-SEC analysis

The Mw distribution chromatograms of water extractable compounds (prepared as described in chapter 2.3.3.) of bran treated for 4 and 24 h at 40 and 90% water contents are presented in Fig. 3. After treatment with low enzyme dosage of 97 EU/g and short treatment time (4 h), there were no significant differences between the elution profiles of the samples treated at different water content. However, when longer treatment time or higher enzyme dosage was used, the amount of large polymers eluting before 53 min (corresponding to Mw of >1500 Da) was higher after treatments at 40% than at 90%. In untreated bran, there was a sharp peak of polymers eluting at 52.2 min (corresponding to Mw of 2000 Da), and this peak was still detectable after enzyme and control treatments at 40%, but not after treatments at 90%. The largest polymers (Mw exceeding 50 000 Da) were detected after treatment at 40% without added enzymes.

4. Discussion

In the present work, the impact of water content and xylanase treatment was studied by analysing the physical state of bran-water mixtures, remaining xylanase activity and depolymerisation of water extractable compounds, in relation to AX solubilisation. The solubilisation of AX was evaluated by analysing the changes in the content of water extractable pentose sugars. It is well known that AX represents about 70% of the non starch polysaccharides in wheat bran (Maes and Delcour, 2002), hence, also the pentose sugars are mostly derived from AX. Pentose sugars may also originate from arabinogalactan peptide, which is easily extractable (Fincher et al., 1974). No data is available about the content or presence of AGP in bran, but in wheat flour, the contents of WE-AGP have been reported to be around 0.15–0.38% dry basis (Andersson et al., 1994; Loosveld et al., 1997). As the reported values of AGP in wheat (though available only for flour fractions) are very low

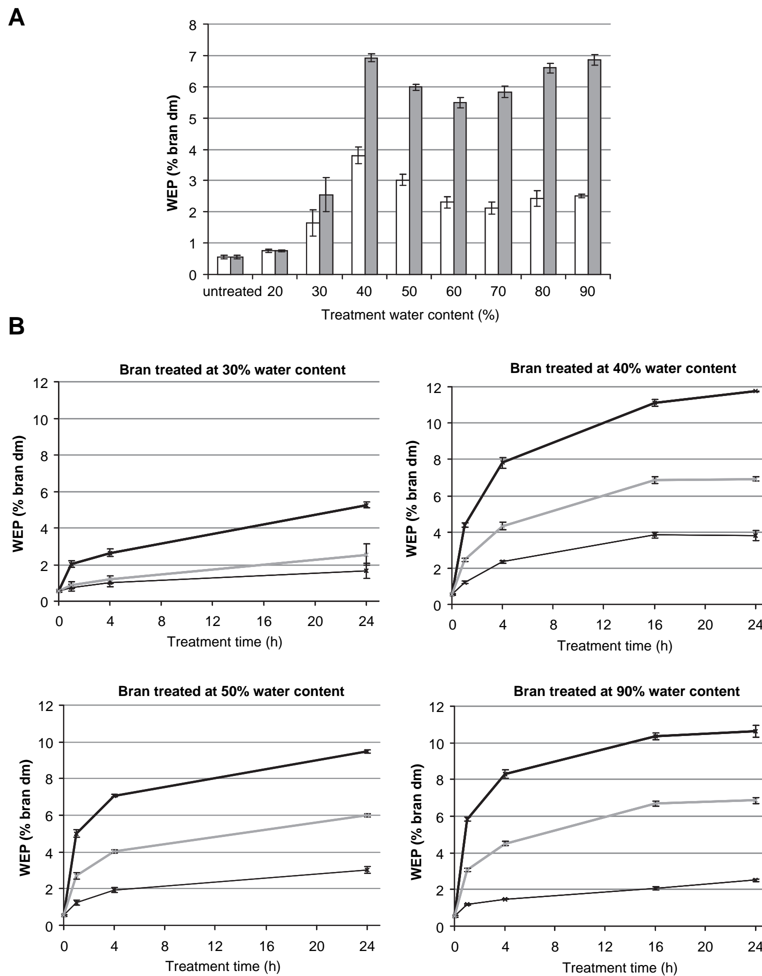

Fig. 2. A) Effect of treatment water content and xylanase addition of 97 EU/g on the content of water extractable pentosans (WEP) of wheat bran after 24 h incubation. □ no added enzyme ■ xylanase 97 EU/g. B) Effect of incubation time and xylanase addition of 97 and 970 EU/g on the content of WEP of bran treated at the water contents of 30, 40, 50 and 90%. The content of WEP was analysed after 1, 4 and 24 h, and at the water contents of 40 and 90%, also after 16 h incubation. The results are expressed as means of duplicate analyses for each bran sample. —— no added enzyme —— xylanase 97 EU/g —— xylanase 970 EU/g.

compared to the level of AX in bran, which has been reported to be in the range of 13–30% in common wheats (Gebruers et al., 2008; Kamal-Eldin et al., 2009), it can be concluded that the increase in the content of WEP, reported in the present study, was due to solubilisation of AX.

Enzymatic solubilisation of wheat bran AX was demonstrated to proceed efficiently even at low water contents. Interestingly, the solubilisation was highest at the water contents of 90 and 40%, whilst at intermediate water contents (50–80%) the solubilisation was lower. However, at the water content of 40%, the solubilisation of AX was most probably enhanced also by other factors than by the hydrolysing activity of the added xylanase.

There are only a few previous publications dealing with the effect of water content on the efficiency of enzymatic modification of bran and AX (Moore et al., 2006; Napolitano et al., 2006; Sørensen et al., 2006). Moore et al. (2006) have successfully treated wheat bran with hydrolytic enzymes at water contents of 30–43% for improving the bioaccessibility of antioxidants. Most of the previous studies, however, have reported decreased enzymatic function at decreased water contents. The yields of enzymatic degradation of AX in a wheat derived fermentation residue were found to decrease as a function of the substrate dry matter level ranging from 2.5 to 10 wt% dm (Sørensen et al., 2006). In a study of enzymatic solubilisation of DF from durum wheat fibre at substrate concentrations of 3.3–33.3%, a concentration of substrate of 6.6% gave the most satisfactory results, whilst at the substrate concentration of 33%, very low enzyme hydrolytic activity was detected (Napolitano et al., 2006). According to the results of several publications collected by Kristensen et al. (2009), it has also been shown that in the enzymatic conversion of lignocellulosic biomass to fermentable sugars, increasing substrate concentration leads to decreased conversion. The main cause of this phenomenon has been suggested to be the inhibition of enzyme adsorption by hydrolysis products (Kristensen et al., 2009), but other possible

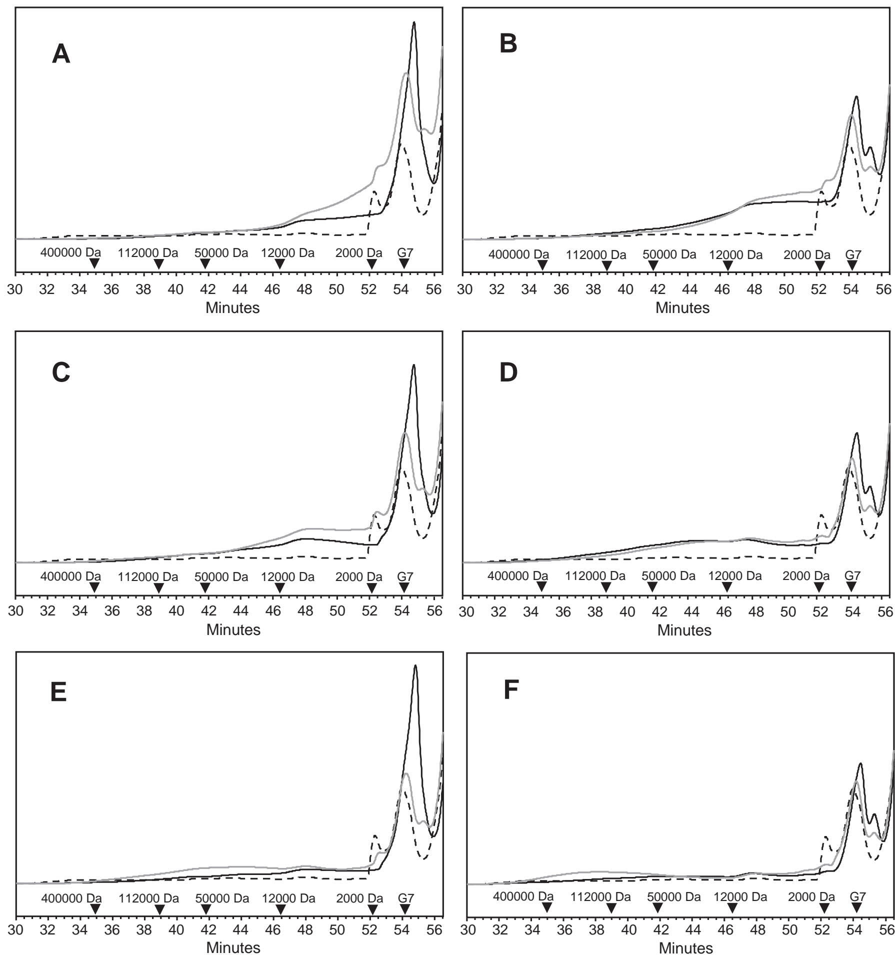

Fig. 3. Mw distribution of water extractable compounds (prepared as described in chapter 2.3.3.) of bran treated with A) xylanase 970 EU/g for 24 h, B) xylanase 970 EU/g for 4 h, C) xylanase 97 EU/g for 24 h, D) xylanase 97 EU/g for 4 h, E) no added enzymes for 24 h, F) no added enzymes for 4 h - - - - untreated bran —— treated at 40% water content ▬▬ treated at 90% water content.

explanations have also been proposed, as reviewed by Kristensen et al. (2009). These previous studies have investigated only the area above the water content of 60%. Similarly, in the present study the solubilisation of AX was decreasing with decreasing water content in the area between 60 and 90% water contents. At even lower water content of 50 and 40%, however, the solubilisation again increased.

When studying the effects of water content in a biological system, it is important to note that water content can rarely be considered as an independent variable, because water affects directly a number of other factors, such as the viscosity and consistency of the studied material. Consequently, in this study the experiments had to be performed in two different kinds of mixing devices because of the very different consistency of the tested samples. The free water present in the bran mixtures at the water contents of 80 and 90% prevented the use of farinograph as a mixing device because of leaking of water. Similarly, the use of a blade mixer for samples of water content less than 80% was not possible because of too high viscosity of the mixtures. Thus, the possible impacts of these two different mixing methods on the AX solubilisation need to be taken into account when interpreting the results.

The solubilisation of AX with added xylanase was highest and approximately the same at water contents of 40 and 90%, but the solubilisation without added enzymes was notably higher at the water content of 40%. Thus, the increase in AX solubilisation at the water content of 40% was evidently caused not only by the action of added xylanase alone, but also by other factors. During the treatments at the water content of 40%, the bran material was transformed to a compact, plastic-like mass. The compact structure of the material was also observed by the measured resistance values. At the water content of 40%, AX was notably solubilised also without added xylanase, which suggests the presence and action of endogenous enzymes in the bran material. However, it is possible that the compact structure of the material at 40% water content might have enhanced the AX solubilisation by physical breakdown of bran cell walls due to shear forces. The degradation of cell wall structures might have increased AX solubilisation, e.g. by causing the breakdown and release of AX molecules that were initially

bound to other cell wall structures, or by facilitating the action of hydrolytic enzymes by exposing new, initially inaccessible AX substrates. In the present study it was calculated that the contribution of added xylanase to the AX solubilisation was 63% at the water content of 90%, while at the water content of 40%, it was only 45%. However, it is difficult to estimate to what extent the solubilisation without added enzymes was caused by the mechanical work input alone, or by the possible synergistic action of both mechanical breakdown of the components and the concomitantly enhanced action of bran endogenous enzymes. It has also previously been postulated that wheat flour AX can be solubilised by disaggregation of AX chains by a temperature increase or mechanical work input (Cleemput et al., 1997; Dornez et al., 2007). Wheat and rye bran AX have also been solubilised mechanically by intensive ball milling process (Van Craeyveld et al., 2009).

The property of bran to absorb water is important in terms of physical properties of bran-water mixtures, and thus, also in terms of AX solubilisation. The approx. WHC of bran was 2.8 g water/g bran dm, which means that bran can hold all the added water up to a water content of 74%. It is likely that the increase in the amount of free water has improved the mass transfer and diffusion of components at the water contents between 70 and 90%, and thus improved the AX solubilisation. At the water contents between 20 and 70%, there was no free water, which reflected the appearance and consistency of the mixtures. Below the water content of 40%, the material was powder-like and AX solubilisation was the lowest. It is presumable that the enzyme diffusion at water contents below 40% is restricted by the absence of a continuous water phase.

Interestingly, the endoxylanase activity was significantly decreased during the treatment at the water content of 40%, whereas at 90% the enzyme activity decreased much less. This was unexpected in view of the degree of AX solubilisation. One possible explanation is that the compact structure of the material and efficient binding of enzyme to the substrate at the water content of 40% may have prevented the extraction of the enzyme from the freeze dried sample in the enzyme activity assay used.

The pH of bran samples did not change remarkably during the 24 h treatments, except for the bran treated at the high water content of 90%. Between 16 and 24 h, the pH of bran treated at 90% decreased from above 6 to less than 5. The decrease in pH indicates the growth and metabolic activity of acid-producing bacteria, such as lactic acid bacteria. It is known that the bran material contains naturally quite high amounts of yeasts and bacteria (Katina et al., 2007). The bran in this study was obtained after grain peeling, which is known to decrease the amount of microbial load of bran (Katina et al., 2007). The relatively high temperature of the treatment (50 °C) was also assumed to further restrict the interference of microbes. However, apparently some microbial growth was present after long incubation times at the high water content. On the contrary, in the treatments at decreased water contents, the low water activity has probably restricted the activity of these microbes, as the pH did not significantly change at the lower water contents. Microbes play an important role in the safety issues of bran processing, and as suggested by this study, water content may also be used as a process parameter to control the growth and metabolic activity of microbes.

The Mw distribution of the water extracted compounds of bran (supposed to mainly be composed of AX) was also affected by the water content of the reaction, especially when long treatment time or high enzyme dosage was used. In the elution profiles of all the samples, the largest peak was eluting after 53 min, corresponding to small oligosaccharides having Mw < 1500. However, in this work the interest was in comparing how the water content affects the Mw profiles of the larger sized molecules eluting before 53 min (Mw > 1500). In many food applications, the preferred xylanolytic reaction is the solubilisation of unsoluble bran AX without intensive depolymerisation of WEAX (Courtin and Delcour, 2002). Both with and without added xylanase, the amount of large polymers was higher after treatments at 40% than at 90%, and the peak of 2000 Da polymers of untreated bran were still left after the treatments at 40% water content, whereas at 90%, the peak vanished. This suggests that although the degree of AX solubilisation was the same both at the low and high water content treatments, the depolymerisation efficiency of xylanase was stronger at the high water content. However, the differences in the Mw profiles may partly result from the physical breakdown of the components at the water content of 40%. The results of the present study indicate that the Mw distribution of water extractable polymers may be affected by the water content of the solubilisation process.

In conclusion, AX could be solubilised at the water content of 40% at least as effectively as at the conventionally used high water content, but at intermediate water contents, the solubilisation was lower. It appears that the texture of the reaction mixture is an important factor in AX solubilisation. The impact of water content on enzyme binding is an interesting topic for further research. Further studies are also needed to confirm the possible effects of mechanical energy input and the role of endogenous enzymes on AX solubilisation. The amount of process water is an important factor in the development of new sustainable enzyme processes, and it is evident that from an ecological and economical point of view, it would be advantageous to be able to produce the same amount of dry product using less water. However, the overall industrial feasibility of a production process depends of course on several factors that may be affected by the water content of the process material. For example, the physical structure of low water content material may pose challenges and require specific process equipment.

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