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Use of an extruder for pre-mixing enhances xylanase action on wheat bran at low water content

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HIGHLIGHTS

- Xylanase action (AX solubilisation and hydrolysis) on wheat bran was studied.
- Impacts of mixing method, water content and bran particle size were studied.
- With xylanase, MW of WEAX was not significantly affected by these variables.
- Extruder enhanced AX solubilisation as compared to blade mixing at low water content.
- Plasticization in extruder probably enhanced xylanase action via improved diffusion.

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ABSTRACT

The aim of the work was to test the hypothesis that at low water content enzyme action on biomass is enhanced when the raw material is in the form of a continuous mass instead of powder/granular form. Effects of two pre-mixing methods, blade-mixing and extrusion, on xylanase action were studied during stationary incubation of wheat bran of different particle sizes, also in comparison with incubation at high water content with continuous stirring. The use of an extruder enhanced arabinoxylan (AX) solubilisation at low water content (<54%), as compared to blade-mixing. AX solubilisation was highest in the high-water stirring treatment, but based on molecular weight, xylanase action on solubilised AX was similar as in the extrusion-aided process. Pre-mixing by extrusion enabled efficient enzyme action at low water content without the requirement for continuous mixing, probably due to the enhanced diffusion by the formation of a continuous mass in the extruder.

1. Introduction

In the extrusion process food ingredients are forced to flow, under mixing, heating and shear, through a die that forms and/or puff-dries the ingredients (Rossen and Miller, 1973). Extrusion technology can be used either to form ready-to-eat foods (snacks, cereals, pasta, confectionery) or to modify food ingredients. A less studied approach is to use extruders as bioreactors for enzymatic processes under elevated temperature, pressure and shear, at moisture levels as high as 70% or more. "Wet extrusion" with feed moisture content above 40% has been possible only since the late 1980s due to developments with twin screw extruders including sophisticated barrel designs, screws and dies (Akdogan, 1999). Compared to traditional stirred-tank reactors, extruders and screw reactors present a cost competitive alternative reactor type for enzymatic modification of biomaterials, especially when targeting dry end products, since the extruders can operate at higher solids content, thus reducing the need for addition and removal of large amounts of water.

The use of an extruder for enzymatic modification has previously been studied mainly for liquefaction of starch by thermostable α-amylase (Linko, 1989; Tomás et al., 1997; de Mesa-Stonestreet et al., 2012). The impact of water content has also been studied, and in most cases the conversion has been highest at the highest water content studied, typically at 55–70% water content, as reviewed by Linko (1989) and Akdogan (1999), although Tomás et al. (1997) reported maximum starch hydrolysis at an intermediate water content of 60% when studying in the range of 55–65%. However, as starch needs to be gelatinized for efficient liquefaction, these extrusion treatments were typically performed above 70 °C or even above 100 °C. Studies concerning the use of extrusion for enzymatic modification of other cereal materials or for other targets are rare. However, two recent papers reported the use of cell wall degrading enzymes for enzymatic hydrolysis of oat bran β-glucan at a water content of 50% (Sibakov et al., 2013a) and for modification of brewer's spent grain at a water content of 65% (Steinmacher et al., 2012).

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Several biocatalytic and thermomechanical methods and processing conditions have been studied as potential means to modify the properties of wheat bran and its components, since wheat bran is a nutritionally appealing (high in dietary fiber, protein and phytochemicals) and widely available raw material which is currently under-utilised as a food ingredient due to its technological and sensory challenges (Sibakov et al., 2013b). Particle size is an important parameter for the use of bran, affecting both its physiological effects and technological functionality (Hemery et al., 2011; Robin et al., 2012; Zhang and Moore, 1999). Decreasing the particle size by grinding increases the surface area available for reactions. It has been shown that decreasing the particle size of plant materials may enhance their enzymatic hydrolysis (Silva et al., 2012; Niemi et al., 2012; Mahasukhonthachat et al., 2010; Dasari and Berson, 2007). Reduction of particle size can also affect the physicochemical properties of bran, such as water uptake and solubility (Mahasukhonthachat et al., 2010; Zhu et al., 2010), as well as the rheological behaviour of biomass slurries (Dasari and Berson, 2007; Viamajala et al., 2009), which may play an important role in enzymatic processes especially at low water content, when only a limited amount of free water is available.

Solubilisation of the arabinoxylan (AX) of cereal bran by endoxylanases has been shown to modify the technological properties of the bran (Katina et al., 2012; Lebesi and Tzia, 2012). We have previously shown that enzymatic solubilisation of bran AX can be efficiently performed even at low water content (40%) using continuous mixing (Santala et al., 2011, 2012). However, when processing at low water content, i.e. at high consistency, continuous mixing requires a high amount of energy, which may not be feasible in industrial processes. In the previous study (Santala et al., 2011), the enhanced AX solubilisation was related to the formation of a compact plastic mass during the treatment at the water content of 40%, because it resulted in the reduction of bran particle size due to the high shear during the treatment with continuous mixing (Santala et al., 2012). However, it is also possible that the formation of a continuous mass from the granular/powdery material can be used as a means to facilitate enzyme action even without continuous mixing. In the current study the aim was to test this hypothesis by studying the effects of two different pre-mixing and forming methods, blade-mixing and extrusion, on xylanase action during stationary (i.e. without stirring) incubation of wheat bran. The impact of treatment water content and bran particle size on the solubilisation and hydrolysis of AX during the different processes was investigated. Further, the aim was to compare the stationary enzyme incubation at low water content to incubation with continuous stirring at high water content.

2. Methods

2.1. Bran

Commercial wheat bran (Fazer Mill & Mixes, Lahti, Finland) was used as raw material and ground by TurboRotor technology (Mahltechnik Görgens GmbH, Dormagen, Germany) to three different levels of fineness. The particle size distributions of the bran raw materials (Table 1) were determined in triplicate from dry bran dispersions by laser light diffraction (Mastersizer 3000, Malvern, Worcestershire, UK) and calculated from the volumetric distribution of the particles using the Fraunhofer optical model. The heat damage possibly associated with intensive milling treatments could be avoided, since in the grinding technology used the high air throughput and short residence times ensured that the product temperature remained below 45 °C. The dietary fibre (DF) content of the brans (Table 1), was analysed by AOAC method No. 991.43 (Prosky et al., 1988). For the quantification of total AX (Table 1), 0.1 g of bran was mixed with 5 ml of 0.5 M H_2SO_4 and boiled for 30 min and centrifuged, followed by a colorimetric determination (Douglas, 1981).

2.2. Hydration properties

Water holding capacity was determined by the Baumann apparatus as described previously (Santala et al., 2012) using a sample size of 50 mg and measurement time of 30 min.

2.3. Xylanase enzyme preparation

A commercial *Bacillus subtilis* xylanase preparation, Depol 761P (Biocatalysts Ltd., Cardiff, UK), was used for the bran treatments. The activity profile (xylanase 28,660 nkat/g, polygalacturonase 1317 nkat/g, β-glucanase 1625 nkat/g, α-amylase 44 nkat/g, and β-xylosidase 2 nkat/g) of the preparation was previously reported by Santala et al. (2012). According to the manufacturer, the optimum temperature range of the enzyme preparation is 45–55 °C.

2.4. Extrusion-aided and blade-mixed treatments at water contents of 37–60%

The process scheme of the treatments is presented in Fig. 1. For the enzymatic treatments at water contents of 37–60%, the xylanase preparation (in powder form, dosed according to its xylanase activity at 200 nkat/g bran dm) was first mixed carefully with 450 g of dry bran, after which the mixture was pre-conditioned to a moisture content of 20% by adding water slowly while mixing (speed setting 2) with a Kenwood KM300 mixer (Kenwood Ltd., Havant, United Kingdom) with a K-shaped blade for 2 min. Pre-conditioning was also performed for the blank extruder treatments (i.e. without enzyme addition). For the extrusion-aided treatments, the pre-conditioned bran mixture was transferred to the feeding bowl of a co-rotating twin screw extruder (APV MPF 19/25, Baker Perkins Group Ltd, Peterborough, UK) within 20 min and fed to the extruder at a rate of 26 g/min. The barrel temperature was set at 50 °C and it was monitored that the temperature in the barrel remained at 50 °C during all the treatments. The screw speed was 65 rpm. Water was pumped to the barrel at an appropriate rate in order to obtain moisture contents of 37 ± 0.5%, 42 ± 1%, 48 ± 1%, 54 ± 1%, or 60 ± 1% in the

Table 1
Properties of the bran raw materials.

	Unground	Coarse	Fine	Ultrafine
Median particle size (μm)	1001 ± 9	702 ± 59	327 ± 9	81 ± 2
90% of particles < (μm)	2257 ± 16	1873 ± 163	895 ± 57	401 ± 28
10% of particles < (μm)	283 ± 5	127 ± 13	29 ± 1	10 ± 1
Total DF (% bran dm)	48.0	48.9	47.9	48.4
Soluble DF (% bran dm)	3.1	3.5	4.1	4.6
Total AX (% bran dm)	20.6 ± 0.4	20.5 ± 0.3	20.3 ± 0.6	20.6 ± 0.2
WEAX (% bran dm)	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.8 ± 0.1
Water holding capacity(g/g bran dm)	3.7 ± 0.2	3.7 ± 0.2	3.7 ± 0.1	3.3 ± 0.1

The results are expressed as means ($n = 4$) ± standard deviation.

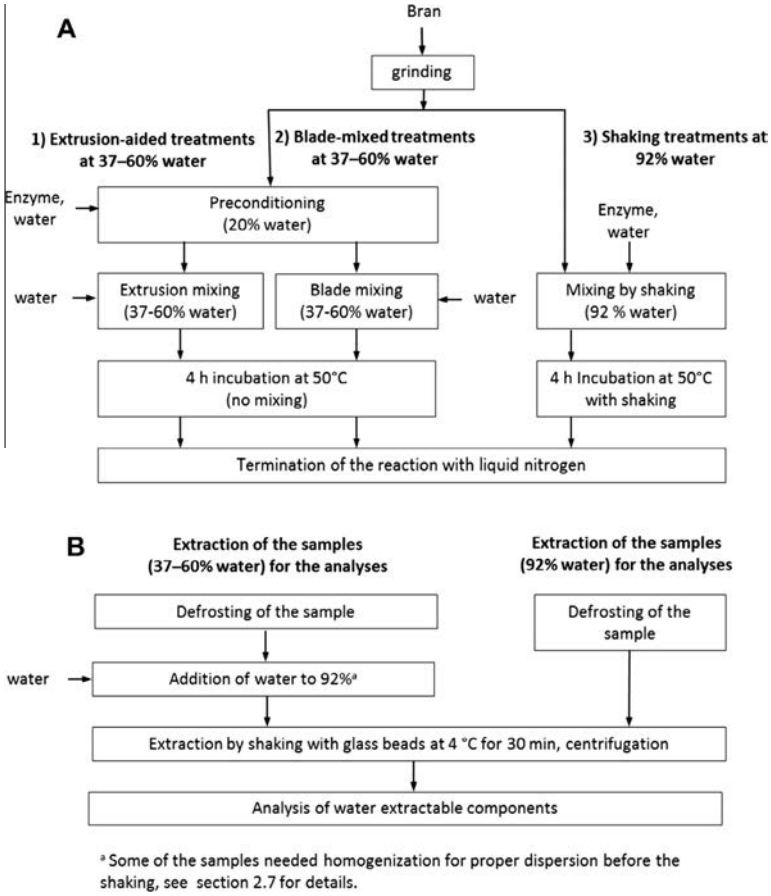

Fig. 1. Process scheme of the bran treatments (a) and extraction procedure (b) for the subsequent analyses.

extrudate. The residence time inside the barrel was about 3 min. Samples were collected from the die exit (diameter 3 mm, each sample was collected for 2 min, corresponding to approx. 20.8 g bran dry matter per sample), after which they were either frozen immediately using liquid nitrogen, or incubated further in sealed containers at 50 °C for 4 h and then frozen using liquid nitrogen.

For the treatments without extruder treatment (hereafter referred to as 'blade-mixed treatments') at water contents of 37–60%, the pre-conditioned bran mixture was pre-heated in a heat chamber at 55 °C for 12 min, after which it was brought to the intended moisture content by adding pre-heated (55 °C) water by spraying while mixing with a Kenwood mixer (K-blades, speed setting 2) for 3 min at 55 °C. The mixture was divided into samples which were either frozen immediately using liquid nitrogen, or incubated further in sealed containers at 50 °C for 4 h and then frozen using liquid nitrogen. Extrusion-aided and blade-mixed treatments were performed in duplicate.

2.5. Incubations at water content of 92%

Bran (3.00 g of bran dm) and the xylanase preparation (in powder form, 200 nkat/g bran dm) were weighed in tared 50 ml centrifuge tubes. Pre-heated water (50 °C) was added to reach a water content of 92%. The sample was then mixed by vortexing and incubated for 4 h with continuous shaking (120 rpm) at 50 °C, after which it was frozen using liquid nitrogen. For the treatments with the lower xylanase dose (20 nkat/g bran), the enzyme was added in the form of a solution (600 nkat/ml, prepared by dissolving the powdered xylanase preparation in distilled water at room temperature by magnetic stirring for 30 min, followed by centrifugation) for more precise dosing. Treatments were performed in duplicate.

2.6. Moisture content analysis

The moisture content in the untreated and ground bran was analysed by oven drying a sample of 1–2 g at 130 °C for 1 h. For the treated samples (with moisture content up to 60%), the sample size was 4–7 g and it was freeze dried (in the tared moisture dish) prior to the oven drying (130 °C, 1 h) in order to ensure complete removal of moisture. The analysis was made in triplicate.

2.7. Extraction of bran samples for the AX analyses

The frozen bran samples were extracted in cold water after rapid defrosting in order to avoid additional enzymatic action during the extraction process. The required quantity of frozen or zuntreated sample (except for the samples incubated at 92% water content), corresponding to 3 g of bran dm, was weighed into a

tared 50 ml centrifuge tube and stored at 4 °C (max. 1 h) until the extraction. The sample was diluted by a factor of 12 (resulting in a total volume of approx. 36 ml and a sample concentration of 8%) using ice-cold distilled water. The actual bran concentration in the extraction mixture was calculated by weighing the tube with diluted sample after water addition. Approximately 24 glass beads (size 4.5–5.5 mm) were added and the sealed tube was shaken in a reciprocating shaker at 4 °C for 30 min. If the sample was not properly suspended with this procedure (due to compact consistency or unhydrated clumps in fine bran treated at a water content of 37% and ultrafine bran treated at water contents of 37% and 42%), the sample was homogenised (10 s, 10,000 rpm with a SilentCrusher M, Heidolph Instruments, Schwabach, Germany) before the extraction. It was confirmed with suitable pre-tests that the use of homogenisation did not increase AX solubilisation of those samples that were completely suspended by the basic procedure. After centrifugation, the supernatant was boiled for 20 min and recentrifuged. The supernatant was stored at −20 °C until analysis. The samples incubated at 92% water content were defrosted in a water bath for about 30 min ensuring that the sample temperature did not exceed 4 °C, after which glass beads were added and the extraction was continued as described above.

2.8. Quantification and molecular weight (MW) distribution of AX

The contents of AX in the water extracts (hereafter referred to as water extractable AX, WEAX) were determined by a colorimetric phloroglucinol method (Douglas, 1981) using xylose as a standard. Free pentose sugars were corrected by a factor of 0.88 to anhydro sugars. For the analysis of the apparent MW distribution of WEAX, other poly- and oligomeric compounds were removed from the water extract by an enzymatic procedure, followed by EtOH precipitation, using a method modified from that of Andersson et al. (2009). All enzymes were obtained from Megazyme International Ireland. First 1.5 ml of the sample water extract was diluted with 1.5 ml of 0.16 M phosphate buffer (pH 6.5). 12.5 μl of α-amylase (from *Bacillus licheniformis*, 3000 Units/ml on Ceralpha reagent, 40 °C, pH 6.0), 50 μl of lichenase (*B. subtilis*, 1000 U/ml on barley β-glucan, 40 °C, pH 6.5) and 12.5 μl of protease (Subtilisin A from *B. licheniformis*, 300 Units/ml on casein, 40 °C, pH 7.0) were then added. The sample was incubated at 60 °C in a shaking water bath for 70 min and cooled down in an ice-water bath. The complete hydrolysis of interfering oligosaccharides was ensured by a further incubation with β-glucosidase, amyloglucosidase and fructanase. The pH of the supernatant was first adjusted to 4.3 ± 0.1 with 0.325 N HCl, after which 75 μl of β-glucosidase (*Aspergillus niger*, 40 U/ml on p-nitrophenyl β-glucoside, 40 °C, pH 4.0), 7.5 μl of amyloglucosidase (3300 U/ml on soluble starch, 40 °C, pH 4.5) and 75 μl of Fructanase Mixture (exo-inulinase 1100 U/ml on kestose, 40 °C, endo-inulinase 95 U/ml on fructan, 40 °C) were added and the sample was incubated at 50 °C in a shaking water bath for 35 min. After boiling for 20 min and centrifugation, WEAX was separated from smaller proteins and hydrolysed contaminating sugars by EtOH precipitation, effected by carefully mixing 1.0 ml of the supernatant with 2 volumes of 95% ethanol. After 75 min in an ice-water bath, the precipitate was collected by centrifugation and dissolved in 2 ml of distilled water by heating in a boiling water bath for 20 min with occasional mixing. The dissolved sample was filtered through a 0.45 μm filter and analysed by HP-SEC. The liquid chromatograph with Alliance 2690 separation module and M-2414 refractive index detector consisted of three columns (7.8 × 300 mm) of 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 1,660,000 to 5900 Da and maltopentaose were used for calibration. Average MW of the sample was calculated using a 3rd order calibration curve between 32.7 min (the elution point of the largest standard) and 53.0 min (corresponding to a MW of approx. 2 kDa). The amount of WEAX in the HP-SEC sample of treated brans was also analysed by the colorimetric phloroclucinol method (Douglas, 1981). The efficiency of the sample pretreatment was confirmed by HP-SEC, which showed that with the enzymatic procedure used, commercial wheat starch (Fluka Chemie, Buchs, Switzerland, dissolved in 0.08 M phosphate buffer, pH 6.5, at a concentration of 3%), barley β-glucan (Megazyme International Ireland, dissolved at a concentration of 0.5%), and fructan/cellulose mixture (Megazyme International Ireland, dissolved at a fructan concentration of 0.5%), were hydrolysed to oligosaccharides smaller than 2 kDa, whereas the MW of commercial wheat AX (Megazyme International Ireland, dissolved at a concentration of 0.5%) remained unchanged as compared to the AX solution before the enzymatic treatment.

2.9. Particle size determination of treated bran samples

For the analysis of particle size distribution, the treated bran samples were defrosted and dispersed in water as for the extraction of samples for AX analyses (diluting bran with water by a factor of 12 and shaking with glass beads for 30 min), with the exception that the procedure was performed at room temperature. The particle size distributions of the bran–water suspensions were measured by laser light diffraction (Mastersizer 3000, Malvern, Worcestershire, UK) and calculated from the volumetric distribution of the particles using the Fraunhofer optical model.

2.10. Statistical analysis

All bran treatments were made in duplicate, and each sample was analysed at least in duplicate. Thus all the results were calculated as means of at least four analysis results. Data were subjected to analysis of variance using IBM SPSS Statistics 19 (IBM Corporation, Somers, NY, USA), and significant differences ($P < 0.05$) between individual means were identified by the Tukey's test.

3. Results and discussion

3.1. Impact of pre-mixing method on the formation of bran–water mixture and on AX solubilisation at low water content

The impact of two pre-mixing methods, blade-mixing and extrusion at ambient temperature, on xylanase action during 4 h stationary incubation of coarsely (Dv50 = 700 μm) and ultrafinely (Dv50 = 80 μm) ground wheat bran at different water contents was evaluated by analysing the amount of WEAX formed (Fig. 2). The level of WEAX after the treatments varied between 2.6 and 6.3% of bran dm, corresponding to a degree of solubilisation of 13–30% of the total AX content of the bran. The solubilisation of AX was in both brans higher after the extrusion-aided treatments than after blade-mixed treatments at the same water content (Fig. 2a). The greatest difference between the treatments was observed at water contents below 54%: for example, after the blade-mixed treatment of coarsely ground bran (Dv50 = 700 μm) at the lowest water content studied (37%), the amount of WEAX was 2.6% of bran dm, whereas after the extrusion-aided treatment the corresponding value was 4.5% (Fig. 2a).

With the coarsely ground bran, the solubilisation of AX increased rather linearly with the increase of water content in the blade-mixed treatments, and no significant differences in the physical form (moist granular material) Fig. S1, Supplementary information)

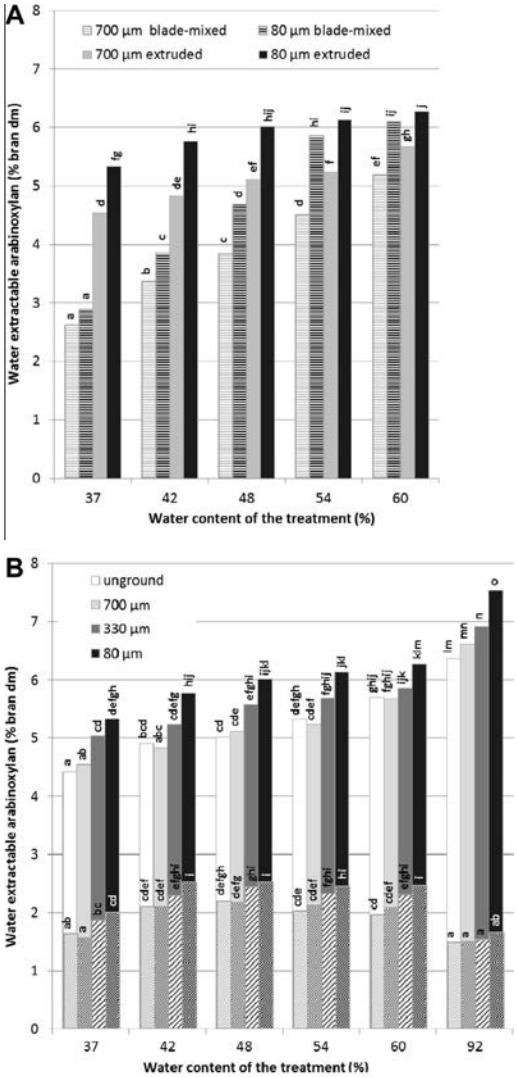

Fig. 2. The amount of water extractable arabinoxylan after blade-mixed and extrusion-aided xylanase treatments (including 4 h stationary incubation) at water contents of 37–60% with coarse and ultrafine bran (a), and after the extrusion-aided and shaking treatments with brans of different mean particle sizes (b). In b the long columns represent samples treated with xylanase and the small columns represent samples without added enzymes. The results are expressed as means (n = 4–8). Values marked with different letters within a set of corresponding samples (in a all samples were analysed in one set; in b the samples with xylanase were analysed separately from the samples with no added enzymes) are significantly different ($P < 0.05$).

of the bran–water mixtures were observed. At a water content of 60%, the level of WEAX after the blade-mixed treatment (5.2% of bran dm) was close to that of the extrusion-treated bran (5.7%). However, in the case of the fine bran, there was a notable increase in the AX solubilisation between 48% (WEAX 4.7% of bran dm) and 54% (WEAX 5.9%) water content, when the AX solubilisation was as high as that of the extrusion-processed bran (6.1%). At a water content of 54%, the bran–water mixture of the ultrafine bran formed a continuous, plastic mass during the 3 min blade mixing, whereas at 48% the mixture remained in the form of a moist granular powder (Fig. S1). This suggests that the formation of a continuous mass (plasticization) might have enhanced the action of the enzyme. The enhanced enzyme activity in the extrusion-aided treatments below a water content of 54%, as compared to the blade-mixed treatments at the same water content, might similarly be due to the formation of plastic mass by the pressure and mechanical shaping in the extruder. In the extrusion-aided treatments the bran mixture was forced through a die after intensive mixing in the extruder barrel, causing the formation of uniform 'sticks' of moist bran mass (Fig. S1). It has also previously been suggested that the reduction of enzyme action at low water content is related to the rheological properties and physical form of the biomass and its polymers, which in turn is related to the state of water in the mixture (Viamajala et al., 2009; Roberts et al., 2011). Viamajala et al. (2009) pointed out that at high solids concentrations the absorption of water within the biomass particles may result in the absence of continuous free water phase, causing the bulk to behave as a wet granular material as portions of the "void" volume contain air rather than liquid. Based on their visual observations, this occurred at insoluble solid concentrations of 30–40% (w/w), corresponding to water contents of 60–70%. Once there is no free water in the system, the material becomes difficult to shear and mix uniformly (Viamajala et al., 2009). The results of the current study suggest that it might be possible to change the granular structure of the material to a continuous mass by using extrusion, without increasing the water content. The continuous form of the material probably enhanced enzyme action by improving diffusion, which is considered a major factor affecting enzymatic reaction rates especially at high solids concentration (Lavenson et al., 2012).

3.2. Impact of high water content and continuous shaking on AX solubilisation

Solubilisation of AX in a conventional high-water (92% in the current study) system with shaking was also investigated and compared to the low water content (⩽60% in the current study) treatments (Fig. 2b), since hydrolytic enzymatic reactions are typically performed at high water content due to the expected reduction of enzymatic hydrolysis at higher solid concentrations. High-solids (i.e. low-water) enzymatic hydrolysis have been roughly defined to take place at the solids content where significant amounts of free liquid are not present (Hodge et al., 2009). The water holding capacity of the bran used in the current study was 3.3–3.7 g water/g bran (Table 1), which corresponds to water contents of 77–79%. Thus, the extrusion-aided treatments performed at water content of 60% and below can be defined as "high-solids" or correspondingly "low-water" treatments.

The solubilisation of AX with xylanase was higher in the high water content treatment (WEAX 6.4% of dm for coarsely ground bran) than in the corresponding low water treatments in the extruder (4.4–5.7% at water contents of 37–60%). On the other hand, efficient mixing and mass transfer are generally considered to be essential for the efficient performance of enzymatic reactions (recently reviewed by Lavenson et al. (2012)), and in that respect even higher differences would be expected because of the lack of stirring during the low water content incubations. Probably the efficient initial mixing was sufficient to facilitate the enzyme action during the extrusion-aided treatment. The impact of mixing on biomass hydrolysis at high solids concentration (15–30%) was studied by Roche et al. (2009), who compared small-scale enzymatic saccharification vessels with three different mixing mechanisms: shaking, gravitational tumbling and hand stirring. They reported that effective initial mixing to promote good enzyme distribution and continued, but not necessarily continuous mixing is necessary in order to facilitate high biomass conversion

rates. On the other hand, very high shear rates might induce inactivation of enzymes (as reviewed by van der Veen et al., 2004), and it is possible that the lower AX solubilisation in the extrusion-aided process as compared to the high-water treatment was partly caused by the enzyme inactivation.

Contrary to the treatments with added xylanase, in the blank treatments with no added enzymes the solubilisation of AX was notably higher in the extrusion-aided processes at the water contents of 42–60% than in the high water content (92%) treatments (Fig. 2b). For example, in case of the ultrafine bran, the level of WEAX after these treatments was 2.5% bran dm, whereas at high water content the level was only 1.7%. The solubilisation of AX was most probably caused by the action of endogenous hydrolytic enzymes of the bran material (Dornez et al., 2009), and the results suggest that low moisture content could be favourable for the solubilisation of AX by endogenous bran enzymes. This would be logical, as the natural environment of the endogenous enzymes is not necessarily highly aqueous. It could also be speculated whether it is possible that the activity of enzyme inhibitors, known to be present in bran material (Jerkovic et al., 2010), could have been reduced by the low water content.

3.3. Impact of bran particle size on bran processing with xylanase

The impact of bran particle size on the solubilisation of AX was studied using brans ground to four different mean particle sizes (1000, 700, 330 and 80 μm). The analysis of soluble and insoluble DF content showed that the total DF content was similar in all the brans (48–49% of bran dm), whereas the level of soluble DF increased with decreasing particle size (from 3.1% to 4.6%), which is often observed after intensive milling (Hemery et al., 2011; Zhu et al., 2010). Similarly, the total AX content was similar in all the brans (20.3–20.6% of bran dm), whereas the content of WEAX increased with decreasing particle size (from 0.6% to 0.9%). The reduction of bran particle size also enhanced the solubilisation of bran AX during all treatments, but the impact was rather small (Fig. 2). For example, at a water content of 48% after the extrusion-aided treatments with xylanase, the WEAX content varied from 5.0% (of unground bran dm) to 6.0% (ultrafine bran). In most points studied, the differences between different brans were statistically significant only when comparing the ultrafine bran to the coarse and unground bran. It was also tested whether the high xylanase dose or the use of glass beads in the extraction method used in the AX assay could have hidden the impact of the particle size on AX solubilisation, but the trend (only a rather small increase in AX solubilisation with reduction of bran particle size) was also the same when a 10% enzyme dose was used in the shaking treatment and the extraction was performed without addition of the glass beads (data not shown).

Several studies have reported that the reduction of particle size of plant-based substrates enhances the efficiency of enzyme action (Silva et al., 2012; Niemi et al., 2012; Mahasukhonthachat et al., 2010; Dasari and Berson, 2007). However, to our best knowledge, the impact of particle size on enzyme function over a wide range of water contents, or together with different processing or mixing methods, had not previously been studied. The present results showed that the impact of bran particle size on enzyme function was similar in all the processing methods and water contents studied, and it was also similar when no added enzymes were used. The only notable exception was in case of the blade-mixed treatments at a water content of 54%, when the difference between the ultrafine and coarsely ground bran was higher than in the other water contents. The enhanced AX solubilisation of the ultrafine bran was most probably caused by plasticization of the ultrafine bran mixture in these conditions (Fig. S1), as already discussed. The higher susceptibility of the ultrafine bran to form continuous mass might be explained by its lower water holding capacity (Table 1). When material binds less water, more water remains to act as a plasticizer. It was also noted that reduction of particle size caused a reduction in the torque values of the extruder, especially at water contents of 54–60% (data not shown), indicating a reduction of viscosity with decreasing bran particle size. Dasari and Berson (2007) and Viamajala et al. (2009) also reported a reduction in viscosity as a result of decreasing particle size. Dasari and Berson (2007) suggested that the reduction in viscosity due to smaller particle size may allow for higher solids loading (i.e. lower water content) and thus reduced reactor sizes in large-scale processing. From the results of the current study it can also be concluded that reduction of bran particle size can be used as a means to maintain AX solubilisation when decreasing the water content. For example, when using the coarse bran (Dv50 = 700 μm), the level of 5.7% of WEAX was reached at a water content of 60%, whereas in case of the ultrafine bran this level was already reached at a water content of 42% (Fig. 2b).

In our previous study in which we studied the solubilisation of AX with xylanase at water contents of 20–90% using continuous mixing, the solubilisation was highest at 40% and 90% water contents (Santala et al., 2011). Reduction of particle size due to the high shear during the treatment with continuous mixing was considered to be one explanation for the enhanced solubilisation at 40% water content (Santala et al., 2012). In the current study, particle size measurement of the extruded sample before incubation showed that the particle size of the coarse bran had decreased during the extrusion, especially at the low water content (Table 2). In the vessel treated samples the particle size was 892–938 μm after the blade mixing (before incubation) at all water contents analysed, whereas in the extruded sample the particle size was 348 μm at a water content of 37%, and 770 μm at a water content of 60%. Reduction in particle size was presumably due to the higher torque (data not shown) exerted on the bran mixture at low water content. Particle size was reduced similarly or even slightly more in the samples with no added enzyme (data not shown), probably due to the lack of lubricating effect of the AX solubilisation caused by the added enzymes. The mechanical particle size reduction in the extruder could have enhanced the AX solubilisation. However, the reduced particle size of the coarse bran was still much higher than that of the blade-mixed ultrafine bran (84–85 μm), which showed lower AX solubilisation after blade-mixed treatment than the coarse bran in the extruder. This confirms that the enhanced AX solubilisation in the extruder-aided treatment was not merely caused by the particle size reduction in the extruder. In addition, the particle size of the blade-mixed ultrafine bran was similar at all water contents studied, confirming that the use of homogenisation in the extraction procedure of the 37% water content samples did not affect the particle size of the bran. The particle sizes of the blade-mixed samples (892–938 μm for coarse and 84–85 μm for ultrafine bran) were slightly higher than those of the untreated brans measured with the dry method (702 and 81 μm), which is probably due to the swelling of the blade-mixed brans by the water of the wet method used for the particle size analysis.

3.4. Impact of different processing methods on the molecular weight of water extractable AX

The apparent weight average molecular weights (MW) of WEAX were analysed from enzymatically purified and EtOH-precipitated AX fraction of selected bran samples (Table 3 and Fig. 3). EtOH precipitation is a generally accepted method for separating WEAX from contaminating protein and mono- and oligosaccharides for the analysis of WEAX MW, due to the lack of a specific detection method for AX. The concentration of EtOH should be low enough to avoid the precipitation of oligosaccharides that would overlap

Table 2
Impact of different pre-mixing methods on the bran particle size at different water contents measured before incubation of the extruded or blade mixed samples with xylanase.

Treatment water content (%)	Coarse bran (d50 = 700 μm) Median particle size (μm)		Ultrafine bran (d50 = 80 μm) Median particle size (μm)	
	Extruded	Blade-mixed	Extruded	Blade-mixed
37	348 ± 13a	902 ± 35d	55 ± 2a	84 ± 5c
48	603 ± 21b	938 ± 31d	76 ± 2b	84 ± 5c
60	770 ± 32c	892 ± 41d	85 ± 3c	85 ± 5c

The results are expressed as means ($n = 6$) ± standard deviation. Values marked with different letters within the same bran type (coarse/ultrafine) are significantly different ($P < 0.05$).

Table 3
Average apparent molecular weight and content of water extractable AX (WEAX) precipitated at 65% EtOH in the brans after different treatments.

	Treatment water content (%)	Coarse bran (d50 = 700 μm) EtOH (65%) precipitated WEAX			Ultrafine bran (d50 = 80 μm) EtOH (65%) precipitated WEAX		
		Average MW (kDa)	Content in bran (% of dm)	% of total WEAX	Average MW (kDa)	Content in bran (% of dm)	% of total WEAX
Untreated bran		158 g	0.4 a	75	143 f	0.6 ab	83
Extrusion-aided treatment without added enzymes	37	131 e	1.3 de	85	133 e	1.7 gh	86
	48	79 d	1.3 d	60	76 cd	1.7 fgh	68
	60	68 b	1.2 cd	57	68 b	1.4 defg	58
Shaking treatment without added enzymes	92	66 b	0.9 bc	57	68 bc	1.1 cd	67
Extrusion-aided treatment with xylanase	37	51 a	1.1 cd	25	52 a	1.7 efgh	32
	48	52 a	1.4 def	27	51 a	1.9 h	31
	60	56 a	1.4 def	24	52 a	1.9 h	31
Shaking treatment with xylanase	92	51 a	1.9 h	29	48 a	2.7 i	35
Blade-mixed treatment with xylanase	37	69 bc	1.3 d	50	73 bcd	1.8 gh	62
	48	55 a	1.3 def	35	52 a	1.8 h	39
	60	53 a	1.3 d	25	51 a	2.0 h	33

The results are expressed as means ($n = 4$). Values marked with different letters within the same analysis (MW/AX content) are significantly different ($P < 0.05$).

with the unwanted residues at the low molecular size end of the chromatogram. However, with decreasing EtOH concentrations, more of the original WEAX is lost as only WEAX with high MW is precipitated (Swennen et al., 2005). An EtOH concentration of 50–65% has generally been used for separation of WEAX (Andersson et al., 2009; Ganguli and Turner, 2008) and 65% was selected for the current study.

When enzyme was used, the MW of EtOH-precipitated WEAX of the extrusion-processed brans (51–56 kDa) was similar to that of the bran treated at high water content with shaking (48–51 kDa), as well as that of the brans treated by the blade-mixed method at ⩾48% water content (51–55 kDa). In the blade-mixed treatment at 37% water content the MW was higher (69 and 73 kDa with coarse and ultrafine brans), indicating that AX was hydrolysed less efficiently. This is in accordance with the lower level of AX solubilisation during the vessel treatment at 37% water as compared to all other xylanase treatments.

Without added enzyme, the MW of EtOH-precipitated WEAX varied when bran was processed at different water contents by the extrusion-aided process. The MWs of AX in the untreated brans (coarse bran 158 kDa and ultrafine bran 143 kDa) were very close to the values obtained by Zhang et al. (2011) for wheat bran WEAX using a different HP-SEC method (152 kDa). At the lowest water content (37%), the MW of AX of the processed brans (coarse bran 131 kDa and ultrafine bran 133 kDa) did not markedly change from that of the untreated brans, suggesting that at 37% water content, endogenous enzymes of bran material were not able to hydrolyse WEAX. At a higher water content of 48% the MW was already significantly lower (79 and 76 kDa), and at 60% water content the MW was further decreased to the same level as after the shaking treatment at high water content (66–68 kDa).

The results suggest that with added xylanase, the MW was not dependent on the used water content or processing method (extrusion-aided or shaking treatment). By contrast, without added enzymes the hydrolysis of WEAX increased with increasing water content, evidently due to the action of endogenous bran enzymes, whereas the solubilisation of AX was favoured by the low water content process (extrusion-aided treatment), as already discussed. Similarly, in a previous study in which a process with continuous mixing was used, solubilisation of wheat bran AX was higher and less depolymerisation occurred at low (40%) water content as compared to high (90%) water content (Santala et al., 2011). According to Sibakov et al. (2013a), the use of a water content of 90% for enzymatic hydrolysis of β-glucan resulted in rapid breakdown into short oligosaccharides, whereas low water content (50%) enabled a controllable depolymerisation of high MW β-glucan. In the current study, the difference between the impact of water content on the MW of WEAX with and without added xylanase might be due to the differences in the susceptibility of the exogenous and endogenous bran enzymes to water content.

In order to learn how well the determined MW represents the total WEAX of the bran sample, the amount of WEAX in the HP-SEC sample was also analysed (Table 3), as the method used for the MW analysis applies only to the WEAX fraction precipitating at 65% EtOH. The level of WEAX in the HP-SEC samples was 1.1–1.4% of bran dm for coarse bran after all treatments at water contents of 37–60%. After the shaking treatments, the amount was higher when treated with xylanase (1.9% of bran dm), and lower without added enzyme (0.9%). The trend was the same in the case of the ultrafine bran, but the levels were somewhat higher than in case of the coarse bran (Table 3). When compared to the total level of WEAX in the original sample (Fig. 2), the precipitated WEAX

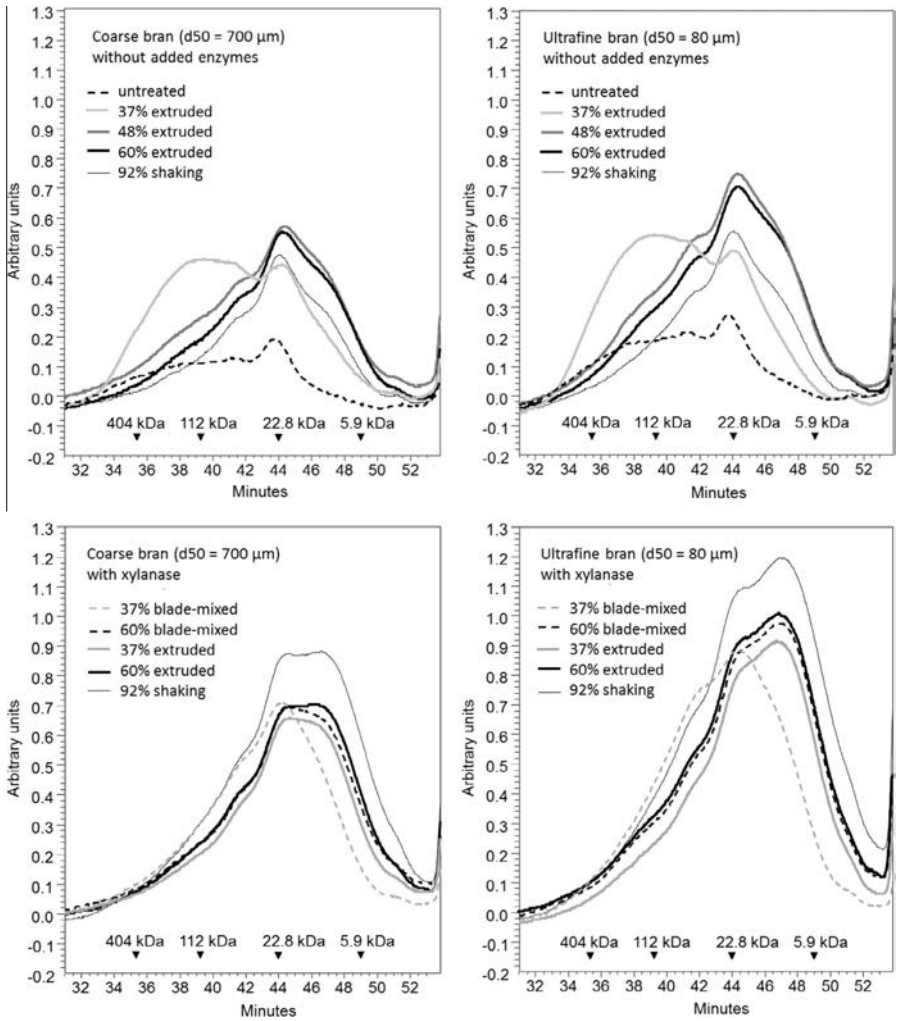

Fig. 3. Molecular weight distribution of water extractable arabinoxylan (precipitated at 65% EtOH) after blade-mixed and extrusion-aided treatments (including 4 h stationary incubation) at water contents of 37–60% and after shaking treatment (water content 92%) with coarse and ultrafine brand.

amounts in the HP-SEC samples corresponded to 24–39% (Table 3) of the total WEAX when bran had been processed with enzyme, regardless of the process used or the water content, except for the vessel treatment at 37% water content, when the precipitation level was higher (50 and 62% for coarse and ultrafine bran, respectively), obviously due to the lower level of total WEAX. The rest of the WEAX were smaller oligosaccharides which did not show in the MW chromatograms. Without added enzymes and in the untreated bran, the analysed MW of WEAX represented as much as 57–86% of the total WEAX (Table 3). The levels of WEAX in HP-SEC samples are not always reported, although they should be taken into account because the level of WEAX in the original sample and the analysed MW are not necessarily comparable as such, especially in the case of enzymatic treatments. It is known that both the level of AX solubilisation (WEAX content as compared to water insoluble AX), as well as the MW of the WEAX are important factors affecting the technological properties of cereal AX (Courtin and Delcour, 2002).

4. Conclusions

An extruder-aided process enabled efficient xylanase action on wheat bran at low water content without the requirement for continuous mixing, probably due to enhanced diffusion by the formation of continuous mass in the extruder. The method may be applicable to other biomass sources as well. AX solubilisation with added xylanase was highest at high-water stirring treatment, but without added xylanase, solubilisation was highest in the extrusion-aided process. Based on molecular weight, xylanase action on solubilised AX was similar in the high-water process and extrusion-aided process. Decreasing bran particle size improved AX solubilisation in rather similar manner in the processes studied.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2013.09.029.

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