PUBLICATION III

Comparison of acid and enzymatic hydrolyses of oat bran β-glucan at low water content

In: Food Research International (52),
pp. 99–108.

Food Research International 52 (2013) 99–108

Contents lists available at SciVerse ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Comparison of acid and enzymatic hydrolyses of oat bran β-glucan at low water content

Juhani Sibakov *, Olavi Myllymäki, Tapani Suortti, Anu Kaukovirta-Norja, Pekka Lehtinen [1], Kaisa Poutanen

VTT Technical Research Centre of Finland, P.O. Box 1000 (Tietotie 2), FI-02044 VTT, Finland

ARTICLE INFO

Article history:
Received 2 December 2012
Accepted 21 February 2013
Available online 4 March 2013

Keywords:
Defatted oat bran
β-Glucan
Soluble dietary fibre
Enzyme-hydrolysis
Acid-hydrolysis
Extrusion

ABSTRACT

The effect of acid- or enzyme-catalysed hydrolysis on partial depolymerisation of β-glucan in oat bran was studied. Hydrolyses were performed at relatively low water content (50% dry matter) using high shear mixing in a twin-screw extruder. The hydrolysed oat brans were extracted with hot water and centrifuged to obtain a water-soluble phase and an insoluble residue. The time-dependent gelling of the water-soluble phase was monitored for 14 weeks at 5 °C. Acid-hydrolysis required a short reaction time (3 min) to depolymerise the β-glucan molecules from their original average M_w of 780,000 to 34,000 g/mol. After acid-hydrolysis, β-glucan had low polydispersity (4.0–6.7). Longer incubation time (3–4 h) was needed for enzymatic depolymerisation of the β-glucan molecules down to 71,000–49,000 g/mol. Enzymatic hydrolysis resulted in high polydispersity (19.0–24.2). The concentration and M_w of β-glucan significantly affected the gelling of hot water extracts. At 1.4–2.0% β-glucan concentration, solutions of β-glucan molecules with M_w > 50,000 g/mol agglomerated rapidly, whereas solutions of smaller molecules (34,000–49,000 g/mol) remained as stable dispersions for longer. Gelling was strongly concentration-dependent and at 1.4 to 1.6% beta-glucan concentration gelling occurred after 7 to 12 weeks of storage, whereas at 1.8 to 1.9% concentration gelling occurred already after 2 weeks of storage.

1. Introduction

Oats are a good source of β-glucan, because they have high initial β-glucan concentration, usually around 4.5–5.5% (Ajithkumar, Andersson, & Åman, 2005; Cho & White, 1993; Saastamoinen, Plaami, & Kumpulainen, 1992). Oat β-glucan is a linear polysaccharide consisting of β-D-glucopyranosyl units, which are joined by (1 → 3)- and (1 → 4)-β-D-linkages. The (1 → 3)-linkages increase the flexibility of the chain and make β-glucan water-soluble (Buliga, Brant, & Fincher, 1986). The main building blocks, cellotriose (DP3) and cellotetraose (DP4), comprise over 90% of the β-glucan molecule. A small proportion of oat β-glucan consists of longer cellulose-like sequences, mainly DP5–DP9, but even up to DP20 (Doublier & Wood, 1995; Lazaridou, Biliaderis, & Izydorczyk, 2003). The average molecular weight (M_w) of β-glucan in untreated oats is usually around 1,500,000–2,500,000 g/mol (Sikora, Tosh, Brummer, & Olsson, 2013). Oat β-glucan has a strong water-binding and viscosity-thickening capacity.

Several studies and meta-analyses have shown that oat β-glucan can reduce LDL cholesterol (low-density lipoprotein) of hyperlipidemic subjects (Brown, Rosner, Willett, & Sacks, 1999; Othman, Moghadasian, & Jones, 2011; Ripsin, et al., 1992). High or medium molecular weight (2,210,000 ≤ M_w ≤ 530,000 g/mol) oat β-glucan has been shown to induce stronger cholesterol lowering capacity compared to low M_w β-glucan (210,000 g/mol) in extruded breakfast cereals (Wolever et al., 2010). High M_w may also be linked to the cholesterol lowering capacity of breads and cookies (Kerckhoffs, Hornstra, & Mensink, 2003). However, Biörklund, van Rees, Mensink and Önning (2005) and Naumann et al. (2006) have shown that low M_w β-glucan (70,000 or 80,000 g/mol) can lower the LDL cholesterol when consumed in beverages. Thus, the food matrix seems to have an effect on the cholesterol lowering, and the role of M_w in predicting β-glucan's cholesterol lowering potency should be further studied.

In low moisture products, such as breads and snacks, much effort is made to retain the high molecular weight (HM_w) of β-glucan (Åman & Andersson, 2008; Duss & Nyberg, 2004). The M_w of β-glucan may be reduced due to endogenous enzyme activity, low pH or high temperature during processing (Åman, Rimsten, & Andersson, 2004; Flander, Salmenkallio-Marttila, Suortti, & Autio, 2007). In high moisture applications the use of HM_w β-glucans is challenging, because they tend to aggregate and form semi-solid or concentrated dispersions. Critical concentration (c^*) is used to describe the concentration at which β-glucan molecules begin to interact with each other. At c^*, the diluted solution turns into semi-diluted, and finally into concentrated solution

Abbreviations: DP, degree of polymerisation; M_w, weight average molecular weight; M_n, number average molecular weight; HM_w, high molecular weight; LM_w, low molecular weight; SDF, soluble dietary fibre.

* Corresponding author at: P.O. Box 1000 (Tietotie 2), FI-02044 VTT, Finland. Tel.: +358 20 722 4080; fax: +358 20 722 7071.
E-mail address: Juhani.Sibakov@vtt.fi (J. Sibakov).
[1] Present address: Senson Oy, P.O. Box 95, FI-15141, Niemenkatu 18, Lahti, Finland.

0963-9969/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodres.2013.02.037

(c^{**}). For HM_w molecules (≈1,000,000–1,500,000 g/mol) the c^* and c^{**} values have been reported to be as low as 0.1–0.4% (Doublier & Wood, 1995; Ren, Ellis, Ross-Murphy, Wang, & Wood, 2003).

To obtain high concentration of β-glucan and yet avoid its aggregation in foods with very high water content, such as beverages, β-glucan needs to be in the diluted region (i.e. high c^* value and low viscosity). To obtain lower viscosity, the M_w of β-glucan must be reduced. This is possible, for example, by high pressure homogenization (Laakso & Lehtinen, 2005), by sonication (Vårum, Smidsrød, & Brant, 1992), by adding ascorbic acid (Kivelä, Gates, & Sontag-Strohm, 2009), by using thermo-mechanical degradation in extrusion (Tosh et al., 2010; Zhang, Bai, & Zhang, 2011), or by acid- or enzyme-catalysed depolymerisation at reduced water content (Kaukovirta-Norja et al., 2009; Lehtomäki & Myllymäki, 2009).

The aim of the current study was to compare the effects of acid- and enzyme-catalysed depolymerisations of β-glucan in an oat bran fraction with high β-glucan content. The stability of hot water-extracted bran hydrolysates at 1.4–2.0% β-glucan concentration was studied during 14 weeks of storage at 5 °C. The objective was to identify the hydrolysis conditions which would enable the production of stable β-glucan dispersions to be used in high-moisture foods, such as beverages.

2. Materials and methods

2.1. Raw materials

The raw material was dehulled, non-heat-treated commercial oat grains from Raisio Oyj (Kokemäki, Finland), the lipid content of which was reduced by supercritical carbon dioxide extraction at NATECO2 GmbH & Co. (Wolnzach, Germany). After the lipid extraction, a β-glucan-enriched fraction was obtained using pin disc milling and air classification at Hosokawa Alpine AG (Augsburg, Germany). First, the pin disc milled oats were separated by air classification into bran and endosperm fractions. Then, the bran fraction was milled and air classified again to obtain a highly enriched β-glucan fraction which contained 33.9% β-glucan, 23.0% protein, 9.2% starch and 4.6% total lipids (Sibakov et al., 2011).

2.2. Acid and enzymatic hydrolyses

The β-glucan-enriched oat bran fraction was first preconditioned by mixing it with 30% water (Fig. 1). The preconditioning ensured that water was evenly absorbed in the matrix. The acid-catalysed hydrolysis was performed with ortho-phosphoric acid (Merck KGaA, Darmstadt, Germany) and enzyme-catalysed hydrolysis with a commercial enzyme preparation (Depol 740L, Biocatalyst Ltd., Wales, UK). The enzyme preparation was produced by *Humicola* spp. and the following activities were detected: Xylanase 17,343 nkat/ml (birch glucurone xylan as substrate, pH 6, 50 °C; Bailey, Biely, & Poutanen, 1992), β-glucanase 6962 nkat/ml (1% barley β-glucan, pH 6, 50 °C; Zurbriggen, Bailey, Penttilä, Poutanen, & Linko, 1990), endoglucanase 614 nkat/ml (1% HEC, pH 6, 50 °C; IUPAC, 1987), β-glucosidase 472 nkat/ml (1 mM 4-nitrophenyl-β-D-glucopyranoside, pH 5, 50 °C; Bailey & Linko, 1990), ferulic acid esterase 52 nkat/ml (pH 5, 50 °C; Forssell et al., 2009), and α-arabinosidase 34 nkat/ml (p-nitrophenyl-α-L-arabinofuranoside, pH 5, 50 °C; Poutanen, Rättö, Puls, & Viikari, 1987).

The hydrolysis was carried out using an APV MPF 19/25 twin-screw extruder (Baker Perkins Group Ltd., Peterborough, U.K.). The feed rate of the preconditioned oat bran into the extruder was 24 g min^{-1}, speed of the twin-screws was 75 rpm, and residence time inside the barrel about 3 min. The temperature inside the extrusion barrel was set to 110–130 °C for acid-hydrolysis and to 50 °C for enzyme-hydrolysis. Water solution of phosphoric acid (8% w/v) or Depol 740L enzyme preparation (50 nkat β-glucanase activity/g oat bran) was fed into the extruder at a rate of 12 ml min^{-1}, resulting in a final water content of 50%.

After the extrusion, the dough-like mass was either ready for subsequent processing (acid-hydrolysis) or incubated in sealed containers at 50 °C for 1–4 h (enzyme-hydrolysis) as shown in Fig. 1. After incubation with the enzyme, the dough-like mass was manually fed into extruder again to inactivate the enzyme. The inactivation was

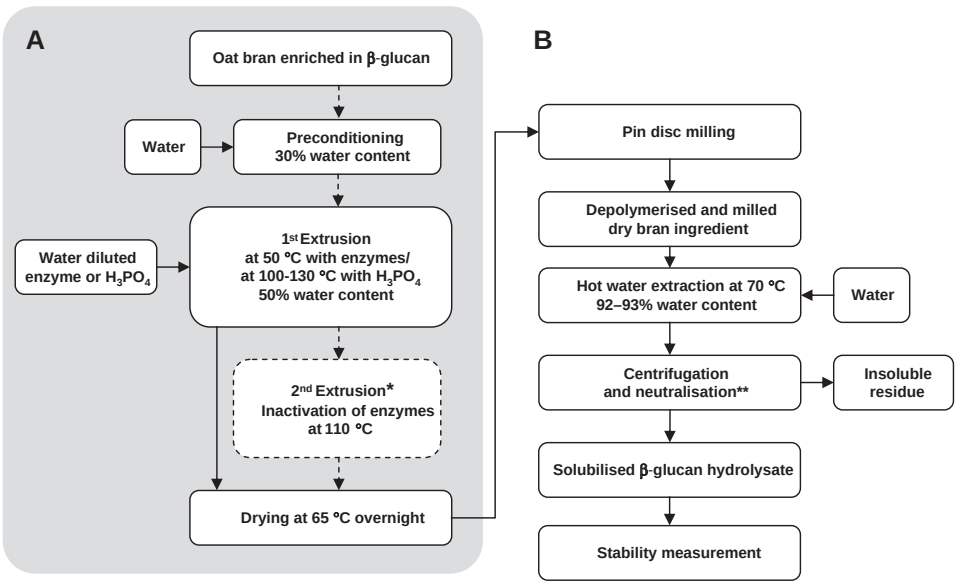


Fig. 1. Process flow chart of the depolymerisation of oat bran enriched in β-glucan. A. Preparation of depolymerised dry oat β-glucan ingredient. B. Extraction of dry ingredient into hot water and separation of solids to yield β-glucan-concentrated beverage base. *The second extrusion was performed after 1–4 h incubation to inactivate the enzyme. **Neutralisation of acid was needed when using phosphoric acid.

performed at 110 °C using 75 rpm speed for the twin-screws, resulting in a residence time of 3–4 min.

2.3. Preparation of the water extractable fractions

After extrusion, the moist, hydrolysed material was spread on stainless steel trays and dried overnight in an oven with recirculation air at 65 °C to avoid microbiological contamination and further depolymerisation of β-glucan. The dried material was first ground in a Wiley cutting mill (Arthur H. Thomas Company, Philadelphia, U.S.A.) and subsequently in a Hosokawa Alpine 100 UPZ-lb Fine impact mill with stainless steel pin discs (Hosokawa Alpine AG, Augsburg, Germany) so that the powder was easier to disperse in water. For the hot water extraction, 66.7 or 83.3 g (d.w.) of dried and milled oat bran material was mixed with 1 l of distilled water at 70 °C. The mixture was stirred for 2 min with a hand-held homogeniser (Heidolph Diax900 Ultra Turrax, Gemini BV, Apeldoorn, The Netherlands), using 12,000 rpm speed. The insoluble residue was separated with a centrifuge (Sorvall RC-12BP, DuPont, U.S.A.), at 4000 rpm (ca. 4000×*g*) for 15 min. The centrifugation was performed at room temperature without cooling to avoid gelling of the water-soluble fraction. The supernatant of acid-hydrolysed oat bran was neutralised from pH 2.1–2.2 to pH 5.0–5.2 with calcium hydroxide (Merck KGaA, Darmstadt, Germany), and the $Ca_3(PO_4)_2$ precipitate formed was separated by centrifugation as described above. Neutralisation was not needed for the enzyme-hydrolysed material, which had a pH of 5.6–5.8.

The supernatants were cooled to 5 °C and stored in a controlled cold room at 5 °C for 14 weeks. The viscosities of the solutions (Pa·s) were measured once a week in a Bohlin Visco 88 Viscometer (Malvern Instruments Ltd., UK) at 5 °C, using eight different shear rates (24–1300 s^{-1}). The solution flasks were mixed well before the analysis. Each time a new sample of 18 ml was taken to be analysed by the viscometer. After the analysis, the sample was thrown away, not returned back to the solution flask.

2.4. Analytical methods

2.4.1. Chemical characterisation

The water-soluble fractions as well as the insoluble residues were dried in a Christ Epsilon 2–25 freeze drier (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). The freeze-dried samples were ground in an Ultra centrifugal mill ZM 200 (Retsch GmbH, Haan, Germany) using a 0.3 mm screen. The total β-glucan concentration was analysed by the spectroscopic method 32–23 (AACC, 2003) using Megazyme β-glucan mixed linkage assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland). The M_w of β-glucan in the hot water extracts was analysed by dissolving the samples at concentration levels of 12 and 8 g/l in 0.2% H_3PO_4 and 200 ppm Na-azide at room temperature overnight with magnetic stirring. The samples were analysed by liquid chromatography, which consisted of an Alliance 2690 separation module and M-2414 refractive index detector. The columns employed were (7.8 × 300 mm) three μHydrogel 2000, μHydrogel 250 and μHydrogel 120 (Waters Inc., Milford, MA, U.S.A.) and Aminex HPX-87H (Bio-Rad, Hercules, CA, U.S.A.) in series at 60 °C. The eluent was aqueous 0.2% H_3PO_4 at a flow-rate of 0.5 ml/min. Injections (50 μl) were made of sample and standard solutions (Suortti, 1993). The linear size-exclusion calibration curve ($r^2 > 0.96$) was constructed on the basis of pullulan standards ranging from 788,000 to 5900 g/mol and malto-oligomers ranging from maltoheptaose to maltose. The system was controlled and calculations were performed with Waters Empower software's GPC option. In principle the software sliced the sample peak into narrow slices. The peak molecular weight value and the area of each slice (i.e. concentration) were calculated by the software. Then the weight average molecular weight (M_w) and the number average molecular weight (M_n) were calculated over the whole β-glucan peak. Polydispersity, a measure of the distribution of individual molecular masses, was calculated as a ratio between M_w and M_n.

Monosaccharides were analysed by extracting 1 g of the cereal sample with 4 ml of cold water (+4 °C) and hydrolysing the water soluble fraction with 1.2 ml of 7.5 N H_2SO_4 at boiling water bath for 2 h. The sugars obtained from the hydrolysis steps and the monosaccharide standards (50 mg/ml; glucose, arabinose, xylose, galactose, mannose) were analysed as their alditol acetates as described by Blakeney, Harris, Henry, and Stone (1983). The dilutions for the standard curves were made from these monosaccharide solutions. Myo-inositol was used as an internal standard (0.5 mg/ml sample). The acetylated monosaccharides were analysed with gas chromatography using an Agilent 6890 GC (Palo Alto, CA, USA) equipped with a flame ionization detector (FID). The column was DB-225 (30 m × 0.32 mm; film thickness 0.15 μm; Agilent). Helium was used as a carrier gas 1.2 ml/min. Split injection (1:3) was performed at 250 °C and the FID-detector was operated at 250 °C. The analytes were separated at 220 °C for 15 min. The monosaccharides were identified according to their retention times and quantitated with standard curve. Free hexose-sugars were corrected by a factor of 0.9 to anhydro sugars, and pentose-sugars by a factor of 0.88. Nitrogen was analysed by a Kjeldahl autoanalyser (Foss Tecator Ab, Höganäs, Sweden), and protein concentration was calculated as N × 6.25 according to the method 46-11A (AACC, 2003). Starch was quantified using Megazyme total starch assay kit according to the method 76–13.01 (AACC, 2003). All chemical characterisations were made in triplicate.

2.4.2. Microscopic analyses

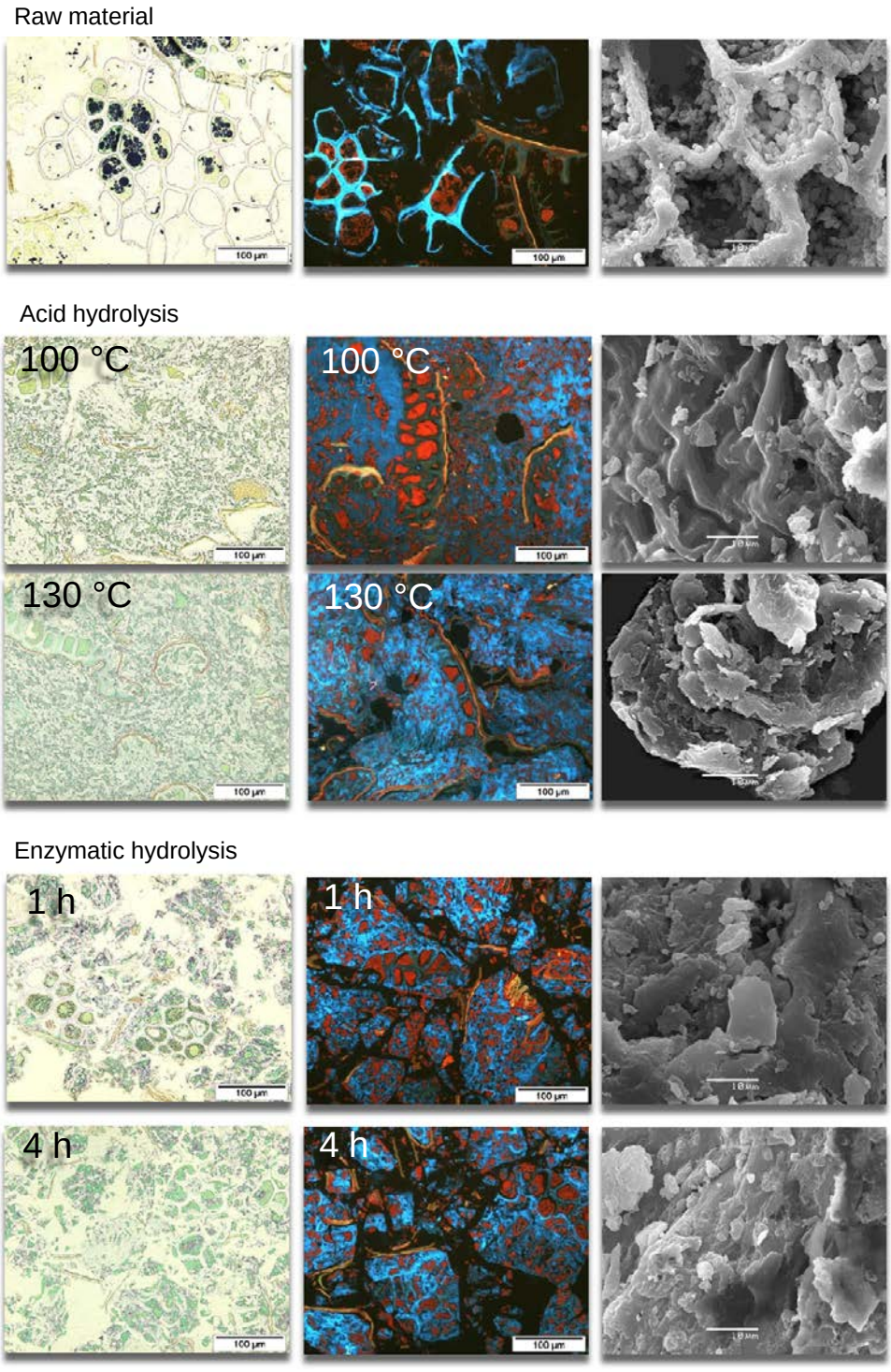
The samples were embedded into agar using a Historesin embedding kit (Leica instruments GmbH, Heidelberg, Germany), and the embedded samples were sectioned (2 μm) in a rotary microtome HM 355 (Microm Laborgeräte GmbH, Walldorf, Germany) using a steel knife. The sections were stained with Acid Fuchsin (BDH Chemicals Ltd., Poole, Dorset UK)/Calcofluor White (Fluorescent Brightener, Aldrich, Germany). Acid Fuchsin and Calcofluor White were used for staining protein red and β-glucan rich cell walls light blue, respectively, and the samples were imaged using exciting light (epifluorescence at 400–410 nm and fluorescence at >455 nm). The samples were then examined under a BX-50 microscope (Olympus Corp., Tokyo, Japan). Micrographs were obtained using a PCO SensiCam CCD colour camera (PCO AG, Kelheim, Germany) and the Cell^P imaging software (Olympus). The electron microscopy was performed using a Jeol 6360 Scanning Electron Microscope (Jeol Ltd., Tokyo, Japan). Prior to imaging, the samples were dried with absolute ethanol and coated with a thin layer of gold atoms using an Edwards Sputter Coater S150B (Massachusetts, U.S.A.).

2.5. Statistical analysis

The results were calculated as means of triplicate analysis results. The data was subjected to analysis of variance using the IBM SPSS Statistics 20 (IBM Corporation, Somers, NY, U.S.A.), and significant differences ($P < 0.05$) between individual means were identified by the Tukey's test.

3. Results and discussion

Low water content (1:1 water and bran) enabled a controllable depolymerisation of HM_w β-glucan into smaller molecules both in acid and enzymatic hydrolyses. Higher water content was also investigated in preliminary experiments. Hydrolysis at 90% water content, using the same concentration of phosphoric acid (8%) or the same enzyme dosage (50 nkat β-glucanase/g oat bran) and at the same temperatures (100–130 °C for acid-hydrolysis or 50 °C for enzymatic hydrolysis) as at 50% water content, resulted in rapid breakdown of β-glucan into short oligosaccharides (data not shown). The results of acid and enzymatic hydrolyses at 50% water content are described and discussed below.


Raw material
Acid hydrolysis
100 °C
100 °C
130 °C
130 °C
Enzymatic hydrolysis
1 h
1 h
4 h
4 h

3.1. Acid-catalysed depolymerisation of β-glucan

Acid-hydrolysis had a great effect on the cell wall structures of the oat bran concentrate. The cell walls were almost completely destroyed as compared to the unhydrolysed raw material. Irrespective of the different hydrolysis temperatures (100 or 130 °C), the microscopic pictures showed similar dispersed distribution of grain compounds (i.e. fragments of cell walls, proteins and starch) in the matrix (Fig. 2).

In the subsequent hot water extraction, 48.2–52.9% of the acid-hydrolysed oat bran material was solubilised and 61.6–68.9% of the total β-glucan in the oat bran fraction was extracted in the water solution (Table 1). The degree of oat bran solubilisation did not differ significantly between the hydrolysis temperatures (100, 120 and 130 °C) and the amounts of added bran (66.7 or 83.3 g/l). However, there seemed to be a trend that higher hydrolysis temperature slightly improved the solubilisation of oat bran. The highest solubilisation of β-glucan (68.9% of the total β-glucan) was obtained when the oat bran was hydrolysed at 120 °C. The solubilisation level was reduced to 63.4–64.3% when the bran was hydrolysed at 130 °C. This was most probably due to harsh hydrolysis conditions, where a small proportion of the β-glucan molecules might have been cleaved down to glucose or even smaller units. However, as the reduction in β-glucan's solubilisation was substantially low, it did not significantly reduce the β-glucan concentrations in the hot water extracts (Table 1).

The composition per dry matter of freeze-dried hot water extracts was: 41.6–45.7% β-glucan, 7.9–8.5% protein and 6.9–8.9% starch. The extracts contained 5.4–5.6% arabinose, 5.8–7.1% xylose, 8.3–8.6% mannose and 52.0–54.1% glucose (Table 2). Similar to the degree of β-glucan solubilisation (Table 1), the β-glucan concentration was highest (45.7%) after the hydrolysis at 120 °C and decreased slightly (to 41.6%) when the hydrolysis was performed at 130 °C. The protein content did not show any clear trend, being lowest (7.9%) after the hydrolysis at 120 °C, but remained at the same level (8.5%) after the hydrolysis at 100 and 130 °C. The starch and xylose concentrations increased when the hydrolysis temperature was raised. However, there were no significant changes in the concentrations of arabinose, mannose and glucose.

The insoluble residues, after the hot water extraction and centrifugation, contained 6.1–6.5% β-glucan, 30.2–35.0% protein and 7.6–11.1% starch, and their monosaccharide composition was: 2.4–3.6% arabinose, 4.4–6.8% xylose, 4.2–4.5% mannose and 18.9–22.6% glucose (Table 2). The differences in β-glucan concentrations were not statistically significant. The protein concentration increased with higher hydrolysis temperature. However, this was not in line with the results obtained from the freeze fried extracts. The reason might be linked to the solubility of different kinds of proteins, but this was not investigated more in detail in this study. Starch and xylose concentrations decreased with the increased hydrolysis temperature. This was in accordance with the increased starch and xylose concentrations in the freeze dried extracts. In addition, glucose concentration decreased slightly between 120 and 130 °C hydrolysis temperatures. As there were no significant differences between the glucose concentrations of the freeze dried extracts, it might be interpreted that some of the glucose molecules were degraded into smaller units at 130 °C.

The depolymerisation of β-glucan was highly dependent on the hydrolysis temperature. The average M_w of β-glucan in the hot water extracts was 110,000; 86,000 and 34,000 g/mol after hydrolysis at 100, 120 and 130 °C, respectively (Fig. 3). The corresponding polydispersity (M_w/M_n) values of the β-glucan molecules were 4.0, 4.3 and 6.7. The low polydispersity values were also detectable as relatively sharp peaks in the molecular weight distributions (Fig. 3).

3.2. Enzyme-catalysed depolymerisation of β-glucan

Enzymatic hydrolysis destroyed most of the cell wall structures, although considerably less severely than acid-hydrolysis. The microscopic analysis showed that some of the cell wall structures still remained intact after 1 h enzymatic hydrolysis, although they were mostly destroyed after 4 h hydrolysis (Fig. 2). In contrast to acid-catalysed hydrolysis, starch particles were still detectable after 1 h enzymatic hydrolysis.

In the subsequent hot water extraction, 29.0–47.1% of the enzyme-hydrolysed oat bran material was solubilised and 44.3–77.9% of the total β-glucan in the oat bran fraction was extracted in the water solution (Table 1). The degree of oat bran solubilisation did not differ significantly after the hydrolysis times of 2–4 h. However, after the 1 h hydrolysis time, a lower solubilisation of oat bran was obtained. The amount of added bran clearly affected the solubilisation of oat bran, being higher with the lower bran proportion (66.7 g/l) compared to the higher bran proportion (83.3 g/l). The reason was most probably related to the high water-absorption capacity of 'mildly' treated oat brans. For example, after being hydrolysed for 1 h with Depol 740L, only 29.0% of bran was solubilised when 88.3 g of oat bran was dissolved in 1 l of hot water. The solubilisation of β-glucan improved significantly when the hydrolysis time was increased from 1 to 3 h. A small reduction in β-glucan's solubilisation between 3 and 4 h hydrolysis time might be explained by a similar cleavage of β-glucan as with the acid. However, the reduction in the level of β-glucan solubilisation between 1 and 4 h hydrolysis time was substantially low, and did not significantly reduce the β-glucan concentrations in the hot water extracts.

The composition per dry matter of hot water extracts of freeze dried material was: 52.2–58.6% β-glucan, 5.0–8.9% protein and 3.8–6.7% starch. The water extracts contained 5.2–6.5% arabinose, 5.6–7.2% xylose, 11.2–11.8% mannose and 59.0–63.7% glucose (Table 2). The β-glucan concentration was reduced with longer hydrolysis time. This could be explained by the cleavage of some β-glucan molecules into glucose. However, the cleavage to glucose should have been low, because the β-glucosidase activity of Depol 740L (3.4 nkat/g oat bran) was almost 15-times lower compared to its β-glucanase activity (50 nkat/g oat bran). This was actually proved with the reference blanks of the β-glucan analysis, where no free D-glucose was detected. Smaller units than glucose were not produced by the enzyme, as might have been the case with acid, because there were no significant differences between the glucose concentrations of freeze dried extracts of enzyme hydrolysed oat brans. The protein concentrations of the freeze dried extracts increased along with the longer incubation time. This was not consistent with the values measured from the insoluble residues, because those did not show any significant differences in protein concentration. Similar to higher temperature in acid hydrolysis, longer hydrolysis time in enzyme hydrolysis resulted in slightly higher concentrations of starch and xylose in the freeze dried extracts.

The insoluble residues after the hot water extraction contained 11.6–16.4% β-glucan, 35.1–35.6% protein and 12.2–14.2% starch, and their monosaccharide composition was: 4.3–5.5% arabinose, 4.6–6.5% xylose, 5.1–5.4% mannose and 26.0–27.6% glucose (Table 2). The clearest difference in the chemical composition of the insoluble residues was in the concentrations of β-glucan. The β-glucan concentration was reduced from 16.4 to 11.6% when the hydrolysis time was prolonged from 1 to 4 h. The significantly higher β-glucan concentration in the insoluble residue of 1 h hydrolysed sample was likely due to the low solubilisation of oat bran after the hot water extraction, and due to the amount of higher M_w β-glucans that did not dissolve into hot water as easily as the lower

Fig. 2. Microscopic pictures of oat bran concentrates: intact oat bran, acid-hydrolysed oat bran (at 100 °C and 130 °C) and enzyme-hydrolysed oat bran (incubated at 50 °C for 1 h and 4 h). First row = column: Light Green/Lugol's iodine staining, showing protein as green and starch as spherical objects in blue or brown. Second row = column: Acid Fuchsin/Calcofluor White staining, showing protein as red and β-glucan rich cell walls as light blue colour. Third row = column: Scanning electron microscopic pictures. Bar = 100 μm (in the first and second rows = columns) and 10 μm (in the third row = column). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Solubilisation of oat bran and β-glucan into liquid phase after acid- and enzyme-hydrolyses and hot water extraction at 70 °C. The results are expressed as mean (n = 3) ± standard deviation. Values marked with different letters within the same row = column are significantly different (P < 0.05).

	Oat bran (g/l of water)	Solubilised oat bran (% of total)	Solubilised β-glucan (% of total)	β-Glucan in water extract (%)
Acid-hydrolysis				
H3PO4, 100 °C	66.7	50.5 ± 0.8 gh	63.8 ± 0.3 d	1.38 ± 0.07 a
H3PO4, 120 °C	66.7	51.3 ± 0.9 h	68.9 ± 0.2 ef	1.56 ± 0.05 ab
H3PO4, 130 °C	66.7	52.0 ± 0.7 h	63.6 ± 0.3 d	1.43 ± 0.06 ab
H3PO4, 100 °C	83.3	48.2 ± 1.1 fg	61.6 ± 0.7 c	1.83 ± 0.11 cd
H3PO4, 120 °C	83.3	51.6 ± 1.1 h	68.9 ± 0.7 e	2.06 ± 0.10 d
H3PO4, 130 °C	83.3	52.9 ± 0.4 h	64.3 ± 0.5 d	1.84 ± 0.04 cd
Enzyme-hydrolysis				
Depol 740L, 1 h	66.7	41.3 ± 0.9 c	71.1 ± 0.7 g	1.55 ± 0.09 ab
Depol 740L, 2 h	66.7	45.4 ± 0.8 de	76.5 ± 1.0 h	1.64 ± 0.07 bc
Depol 740L, 3 h	66.7	47.2 ± 0.6 ef	77.9 ± 0.4 h	1.66 ± 0.08 bc
Depol 740L, 4 h	66.7	45.7 ± 0.8 def	70.2 ± 0.3 ef	1.57 ± 0.06 ab
Depol 740L, 1 h	83.3	29.0 ± 1.7 a	44.3 ± 0.8 a	1.93 ± 0.10 d
Depol 740L, 2 h	83.3	37.6 ± 1.2 b	57.4 ± 0.6 b	1.94 ± 0.12 d
Depol 740L, 3 h	83.3	43.9 ± 0.9 cd	70.5 ± 0.3 fg	2.01 ± 0.06 d
Depol 740L, 4 h	83.3	44.1 ± 0.5 d	67.7 ± 0.4 e	1.94 ± 0.06 d

M_w molecules after longer hydrolysis times (2–4 h). Along with the longer hydrolysis time, there was a small decrease in the concentration of xylose in the insoluble residues. The trend in the concentration of xylose was similar to the results obtained with the acid hydrolysis at increased hydrolysis temperatures. The improved extraction of xylose could be explained by the high xylanase activity in Depol 740L.

The depolymerisation of β-glucan by the enzyme preparation was dependent on the incubation time at 50 °C. The average M_w of β-glucan in the hot water extracts was 218,000; 93,000; 71,000 and 49,000 g/mol after 10 min, 1 h, 3 h and 4 h incubation times, respectively. Compared to acid-hydrolysis, the enzyme-catalysed hydrolysis resulted in wider M_w distributions. The polydispersity value, being lowest with 10 min incubation time, varied between 19.0 and 24.2. The difference between 10 min and 1 h incubations was the most significant, as the average M_w fell below 100,000 g/mol and the proportion of small molecules increased (Fig. 3). The 'tails' at the ends of M_w profiles indicated that part of the β-glucan was depolymerised into shorter oligosaccharides, but their concentrations were not quantified in this work.

3.3. Comparison of acid and enzymatic hydrolyses

The degree and pattern of hydrolysis were different between acid and enzyme-catalysed hydrolyses. The yield of the water extract after acid-hydrolysis (48.2–52.6%) was higher than after the enzymatic hydrolysis (29.0–47.1%). This was probably due to the fact that the acid hydrolysed the grain compounds rather unspecifically (Johansson et al., 2006), whereas the enzyme preparation used, Depol 740L, degraded mainly polysaccharides. In addition, separation of the insoluble residue from the water dispersion of enzymatically hydrolysed oat bran was challenging at 1.9–2.0% β-glucan concentration, due to the high water absorption capacity of the β-glucan molecules with an intermediate M_w (71,000–93,000 g/mol).

Table 2
Monosaccharide compositions of hydrolysed oat brans after water extraction at 70 °C. The water-soluble (extract) and insoluble (residue) fractions were freeze-dried prior to analysis. The concentration of galactose was below the detection limit. The results are expressed as mean (n = 3) ± standard deviation. Values marked with different letters within the same row = column are significantly different (P < 0.05).

	Yield (wt.%)	β-Glucan (g/100 g)	Protein (g/100 g)	Starch (g/100 g)	Arabinose (g/100 g)	Xylose (g/100 g)	Mannose (g/100 g)	Glucose (g/100 g)
Raw material (OBC)	100.0	33.9 ± 0.2 e	23.0 ± 0.2 e	9.2 ± 0.1 f	5.4 ± 0.1 de	6.0 ± 0.1 d	8.6 ± 0.1 b	45.4 ± 0.7 d
Acid-hydrolysed, extract								
Hydrolysed at 100 °C	50.5	42.9 ± 0.6 g	8.5 ± 0.1 d	6.9 ± 0.1 c	5.6 ± 0.1 de	5.8 ± 0.2 d	8.3 ± 0.1 b	52.2 ± 1.3 e
Hydrolysed at 120 °C	51.3	45.7 ± 0.6 h	7.9 ± 0.1 c	7.8 ± 0.1 d	5.4 ± 0.1 de	6.0 ± 0.1 d	8.5 ± 0.3 b	54.1 ± 0.6 e
Hydrolysed at 130 °C	52.0	41.6 ± 0.3 f	8.5 ± 0.2 d	8.9 ± 0.3 e	5.5 ± 0.2 de	7.1 ± 0.3 f	8.6 ± 0.3 b	52.0 ± 1.6 e
Acid-hydrolysed, residue								
Hydrolysed at 100 °C	49.5	6.1 ± 0.1 a	30.2 ± 0.2 f	11.1 ± 0.1 h	3.6 ± 1.3 ab	6.8 ± 0.1 ef	4.2 ± 0.1 a	21.7 ± 0.1 b
Hydrolysed at 120 °C	48.7	6.5 ± 0.2 a	34.5 ± 0.3 g	10.8 ± 0.2 g	2.4 ± 0.3 a	4.4 ± 0.2 a	4.5 ± 0.1 a	22.6 ± 0.2 b
Hydrolysed at 130 °C	48.0	6.1 ± 0.1 a	35.0 ± 0.1 h	7.6 ± 0.1 d	2.4 ± 0.5 a	5.0 ± 0.2 bc	4.4 ± 0.2 a	18.9 ± 0.6 a
Enzyme-hydrolysed, extract								
Incubated for 1 h	41.3	58.6 ± 0.6 l	5.0 ± 0.1 a	4.1 ± 0.1 a	5.2 ± 0.3 de	5.6 ± 0.1 d	11.8 ± 0.8 c	63.7 ± 1.0 g
Incubated for 2 h	45.4	57.3 ± 0.1 k	6.0 ± 0.2 b	3.8 ± 0.2 a	6.0 ± 0.1 ef	6.7 ± 0.2 ef	11.5 ± 0.9 c	61.9 ± 1.8 g
Incubated for 3 h	47.1	56.2 ± 0.2 j	8.6 ± 0.1 d	4.4 ± 0.1 b	6.5 ± 0.5 ef	7.1 ± 0.1 f	11.4 ± 0.6 c	61.7 ± 0.7 fg
Incubated for 4 h	45.7	52.2 ± 1.1 i	8.9 ± 0.1 d	6.7 ± 0.2 c	6.2 ± 0.1 ef	7.2 ± 0.2 f	11.2 ± 0.4 c	59.0 ± 1.0 f
Enzyme-hydrolysed, residue								
Incubated for 1 h	58.7	16.4 ± 0.2 d	35.1 ± 0.1 i	12.2 ± 0.1 i	5.5 ± 0.2 de	6.5 ± 0.2 e	5.3 ± 0.4 a	26.8 ± 0.8 c
Incubated for 2 h	54.6	13.5 ± 0.1 c	35.2 ± 0.2 h	14.0 ± 0.1 k	5.3 ± 0.2 de	5.8 ± 0.1 d	5.4 ± 0.2 a	27.6 ± 0.1 c
Incubated for 3 h	52.9	11.8 ± 0.1 b	35.4 ± 0.1 h	14.2 ± 0.4 k	4.9 ± 0.5 cd	5.1 ± 0.2 c	5.1 ± 0.1 a	26.4 ± 0.4 c
Incubated for 4 h	54.3	11.6 ± 0.1 b	35.6 ± 0.2 h	13.6 ± 0.5 j	4.3 ± 0.1 bc	4.6 ± 0.1 ab	5.4 ± 0.1 a	26.0 ± 0.2 c

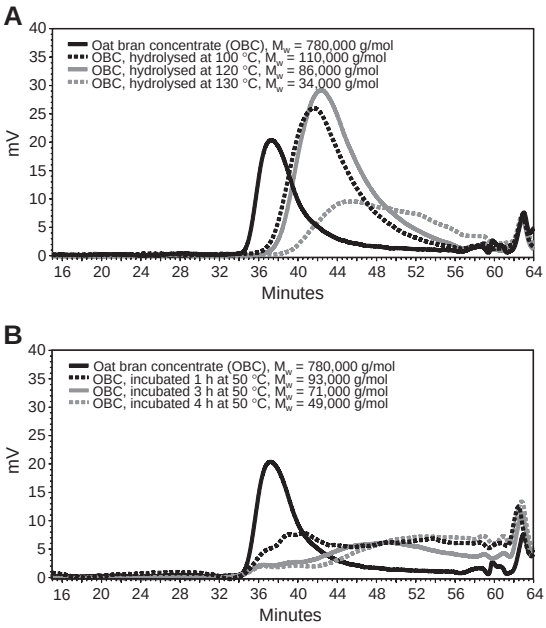


Fig. 3. M_w distributions of the hot water extracts of oat brans after A) acid- and B) enzyme-hydrolysis.

The β-glucan concentrations of freeze-dried extracts were 41.6–45.7 and 52.2–58.6% after acid and enzymatic hydrolyses, respectively. Thus, it was evident that both acid and enzymatic hydrolyses efficiently liberated β-glucan from the oat bran matrix. The β-glucanase in the enzyme preparation used, Depol 740L, was specific for (1 → 4)-β-D-linkages, whereas acid could cleave both (1 → 3)- and (1 → 4)-β-D-linkages (McCleary & Matheson, 1987; Tosh, Wood, Wang, & Weisz, 2004). Therefore the acid-hydrolysed β-glucan molecules may have contained relatively more (1 → 4)-β-D-linkages, meaning that the polymers were more linear. However, this was not fully proved by the current study.

The nature of the enzyme catalysed reaction resulted in wider M_w distributions (Fig. 3) and higher polydispersity values (19.0–24.2) as compared to acid-hydrolysis (4.0–6.7). The side activities of the enzyme preparation may also have affected the results, because the enzyme preparation had several polysaccharide-hydrolysing activities. In earlier studies, for example a very low endo-glucanase contamination in exo-acting cellobiohydrolase II preparation caused considerable changes in the hydrolysis products of β-glucan (Reinikainen, Henriksson, Siika-aho, Teleman, & Poutanen, 1995). However, as Depol 740L contained only low β-glucosidase side activity (around 3.4 nkat/g oat bran), it did not produce free D-glucose and all the β-glucans remained in polymeric or oligomeric form after enzymatic hydrolysis.

3.4. Low water and high substrate vs. high water and low substrate content

Low water content (50% dry matter) maintained the M_w of β-glucan higher than 34,000 g/mol, when using either 8% phosphoric acid at 100–130 °C for 3 min or an enzyme preparation, Depol 740L, with 50 nkat β-glucanase activity per gram of bran at 50 °C for 1–4 h. The same principle as in the current study was used in a patent of Kaukovirta-Norja et al. (2009). They used acid-hydrolysis of oat β-glucan at 45–50% water content. The M_w of β-glucan after acid-hydrolysis of different oat bran preparations at different temperatures and acid concentrations was in the range of 5000–360,000 g/mol. When 8% phosphoric acid was used at 110–130 °C, the M_w of β-glucan was between 37,000 and 135,000 g/mol (oat bran with 22% β-glucan) or between 28,000 and 105,000 g/mol (oat bran with 33% β-glucan). Thus, the results of Kaukovirta-Norja et al. (2009) were in agreement with the results obtained in the current study, in which the M_w of β-glucan was decreased from 110,000 to 34,000 g/mol at temperatures of 100–130 °C. Johansson et al. (2006) showed that at relatively low temperature (37 °C) no degradation of β-glucan was observed with 0.1 M HCl over a 12 h period. By contrast, at high temperature (120 °C) total hydrolysis to D-glucose occurred with 3 M HCl already after 1 h hydrolysis. Hydrolysis with 0.1 M HCl at 120 °C for 1 h produced a range of products, but as the water content was high, the majority of the resulting oligosaccharides had a low M_w (DP 1–7) compared to the molecules in the present study ($M_w \geq 34{,}000$ g/mol, DP ≥ 190). In addition, Tosh et al. (2004) hydrolysed oat β-glucan at high water content (1% dry matter) with 0.1 M HCl at 70 °C for 30–90 min, resulting in fragments with M_w-values from 1,200,000 to 30,000–170,000 g/mol.

The enzymatic hydrolysis at low water content was shown to be highly dependent on the plasticisation phenomenon at 45–50% water content. Similar findings were reported in a patent of Lehtomäki and Myllymäki (2009). They investigated the enzymatic hydrolysis of oat bran at 45–55% water content, using commercial cellulase and α-amylase preparations. They used a twin-screw extruder with a short reaction time (65 s) and an intermediate temperature (65 °C), and controlled the hydrolysis of β-glucan by the dosage of enzymes (17–17,000 nkat β-glucanase activity/g oat bran). The enzymes were inactivated immediately after the short hydrolysis by repeating the extrusion at 95 °C. Unfortunately, the patent did not report the M_w values of the enzyme-hydrolysed oat bran preparations.

Enzymatic hydrolysis at low water content is a complex reaction. In general, enzymes need water for their catalytic activity. Water also influences the structure of enzyme proteins via non-covalent bonding, disrupts the hydrogen bonds, facilitates the reagent diffusion, and influences the reaction equilibrium (Hari Krishna, 2002). Too low water content generally reduces the activity of enzymes, but the minimum hydration level for the enzymatic activity varies considerably between different enzyme preparations. Some enzymes can function even in solvent-free reaction conditions on solid substrates (Hobbs & Thomas, 2007). In addition to oat bran, Moore, Cheng, Su, and Yu (2006), as well as Santala, Lehtinen, Nordlund, Suortti, and Poutanen (2011) and Santala, Nordlund, and Poutanen (2012) have successfully treated wheat bran with hydrolytic enzymes at water contents of 30–90% to improve the bioaccessibility of antioxidants or the solubilisation of arabinoxylans. For example, Santala et al. (2011) showed that the enzymatic solubilisation of arabinoxylan at 40% water content was as efficient as at 90% water content. However, wheat bran is more resistant towards enzymatic hydrolysis than oat bran, because the major part of dietary fibre in oat bran consists of water-soluble β-glucan, whereas most of the fibre in wheat bran is water-insoluble arabinoxylan.

In addition to acid- and enzyme-assisted hydrolyses of β-glucan, Tosh et al. (2010) reported a study of extruded breakfast cereals in which the extrusion temperatures were 181–237 °C and water content was 7.0–18.7%. High specific mechanical energy resulted in the reduction of M_w of β-glucan from 1,930,000 to 251,000 g/mol. It was difficult to compare these results to the current study, because the recipe of the breakfast cereals contained corn flour in addition to the oat bran preparation. The low water content increased the torque during the extrusion, thus leading to much higher specific mechanical energy levels than in the current study. However, it was clearly seen that extrusion without acid or enzymes resulted in lower polydispersity and sharper M_w-distribution peaks compared to the present study (Fig. 3). Zhang et al. (2011) also reported that extrusion can increase the amount of soluble dietary fibre (SDF) in oat bran. Their results showed that the best yield of SDF was obtained with 10% water content. The yield of SDF increased (from 9.9 to 14.2%) when the extrusion temperature was increased from 100 to 140 °C, but somewhat decreased (12.4%

yield of SDF) when the temperature was 160 °C. Unfortunately, this study did not report the M_w-values of β-glucan.

3.5. Stability of depolymerised oat β-glucan dispersions

The intrinsic tendency to form gels and aggregates limits the use of β-glucans in food products with high water content. The size of aggregates has been shown to increase with increasing M_w and concentration (Wu et al., 2006). Similarly, in the current study, the viscosity of the water extracts of the hydrolysed oat brans depended on the average M_w and the concentration of β-glucan (Fig. 4). The hot water extracts prepared from the oat bran after 4 h enzyme-hydrolysis (M_w = 49,000 g/mol) retained their low viscosity only for 2 weeks at 1.9% β-glucan concentration but as long as for 12 weeks at 1.6% β-glucan concentration. Acid-hydrolysis at 130 °C (M_w = 34,000 g/mol), in turn, resulted in elevated viscosities after 3 or 7 weeks at 1.8 or 1.4% β-glucan concentration, respectively (Fig. 4). When the shear rate in the Bohlin 88 viscometer was gradually increased (from 24 to 1300 s^{-1}), lower viscosity values were measured, indicating shear thinning behaviour of the dispersions (data not shown). However, the solutions were considered as Newtonian fluids when their viscosity remained below the detection limit of the viscometer (<0.05 Pa·s).

Even though the average M_w of the acid-hydrolysed β-glucans was lower than after the enzymatic hydrolysis, they resulted in faster and more intensive gel-formation as compared to enzyme-hydrolysed β-glucan. The reason could be that acid-hydrolysed polymer populations had a sharper M_w distribution and more linear chains of β-glucan, which

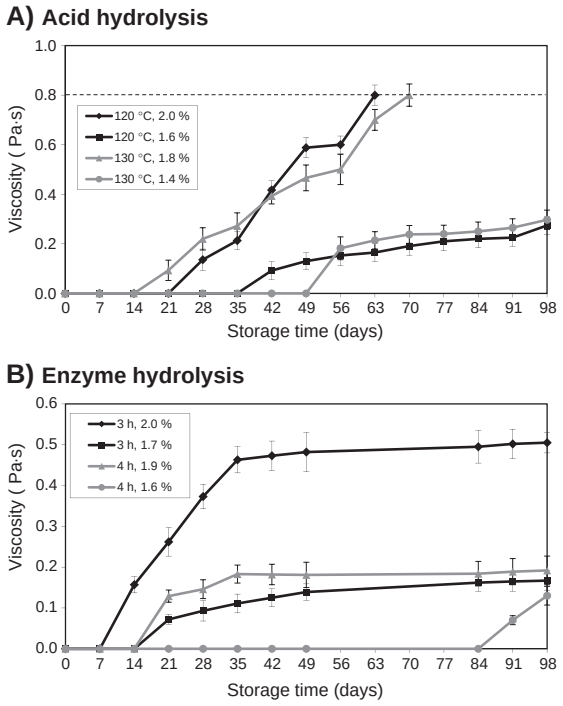


Fig. 4. The viscosity formation of A) acid- (120 and 130 °C) and B) enzyme-hydrolysed (3 and 4 h) oat bran extracts. The viscosity profiles were measured from acid-hydrolysed solutions with 1.4–2.0% β-glucan and from enzymatically hydrolysed solutions with 1.6–1.9% β-glucan. The viscosity values were measured at 24 s^{-1} shear rate in the viscometer. The dashed line (at 0.8 Pa s) shows the measurement limit of the viscometer. The results are expressed as means of duplicate analyses.

easily form aggregates with each other (Doublier & Wood, 1995). The agglomeration phenomenon has also been explained by the intermolecular interactions resulting from the increased mobility of hydrolysed macromolecules (Böhm & Kulicke, 1999; Doublier & Wood, 1995). As the hydrolysed macromolecules are more mobile, they have a greater probability to achieve the proximity of other compounds with regions required for aggregation (Doublier & Wood, 1995; Vaikousi, Biliaderis, & Izydorczyk, 2004).

The HM_w oat β-glucans (>250,000 g/mol) have been shown to form stronger gel networks, which consist of micro-aggregates with better organization, than their LM_w (35,000–140,000 g/mol) counterparts (Lazaridou et al., 2003). However, unhydrolysed HM_w oat β-glucan solutions (>1,200,000 g/mol) have not shown any tendency to form gel (Doublier & Wood, 1995). According to Agbenorhevi, Kontogiorgos, Kirby, Morris, and Tosh (2011), the solution viscosity was higher for HM_w samples when oat β-glucan molecules with different M_ws (142,000–2,800,000 g/mol) were compared at the same concentrations (0.01–8.0% w/v). Increased critical concentration (c^* = 0.25–1.10%) with decreased M_w of β-glucan (from 2,800,000 to 142,000 g/mol, respectively) showed that coil overlap occurred at lower concentrations in the case of HM_w samples (Agbenorhevi et al., 2011).

Similar critical concentrations (c^* = 0.5–2.0%) were also observed by Böhm and Kulicke (1999) for hydrolysed barley β-glucans (M_w range between 375,000 and 40,000 g/mol, respectively). Nevertheless, the solution stability of barley β-glucan differs from that of oat β-glucan. Barley β-glucan has a higher tri- to tetrasaccharide ratio (DP3/DP4) and more ordered cellotriose units compared to oats, and consequently a greater gelling and aggregate-forming tendency (Lazaridou & Biliaderis, 2004; Lazaridou, Biliaderis, Micha-Screttas, & Steele, 2004). Zheng, Hess, Khare, Hilbert, and Deguise (2004) patented an enzymatic method to reduce the M_w of barley β-glucan, based on the cellulase activity in the enzyme preparation. The M_w was preferably between 120,000 and 170,000 g/mol. In large scale, the dry matter concentration in hydrolysis could be up to 18%, which was however much lower than presented in the current study (50% dry matter). Zheng et al. (2004) reported that a 0.75–0.78% w/v solution of 120,000–170,000 g/mol β-glucan in water showed little or no precipitation when stored overnight (16 h) at 4 °C. However, they used a β-glucan ingredient which was highly purified (up to 75–78% β-glucan concentration) prior to the solution stability tests.

Tosh et al. (2004) investigated the ability of acid- (HCl) and enzyme-catalysed (lichenase and cellulase) hydrolyses to produce LM_w β-glucan molecules (31,000–237,000 g/mol). Independently of the hydrolysis method, all 6% oat β-glucan solutions with M_w < 150,000 g/mol formed gels at 5 °C in less than one week. The authors also showed that the time required for a gel to form became shorter in relation to the reduced M_w. These findings were opposite to the results obtained in the present study. It is difficult to compare the results, because the β-glucan concentration (6%) and purity of the β-glucan preparation (92%) were much higher than in the present study (max. 2 and 34%, respectively). Tosh et al. (2004) measured the apparent viscosity of the freshly dissolved 6% β-glucan at 25 °C, and the gel-samples in the rheological measurements were heated from 5 °C (at 5 °C/min) up to the melting temperature of the gel. In addition, they used lichenase enzyme which is known to cleave the (1 → 3)-β-D-linkages of β-glucan, leaving a higher amount of linear (1 → 4)-β-D-linked units in the dispersion, thus enhancing the gelling behaviour of the extracted β-glucan molecules.

In the present study, the oat bran material was extracted with hot water directly after the acid or enzymatic hydrolysis. In consequence, the resulting water extracts also contained other oat-based compounds, such as proteins, starch and arabinoxylan. These components may have prevented the interactions between the regions in the β-glucan molecules which are prone to agglomeration, thus limiting the increase in viscosity. In addition, the polydispersity values in the study of Tosh et al. (2004) were much lower (1.2–1.7) than those in

the present study (4.0–24.2). The oligosaccharides generated by the enzymatic hydrolysis in the present study may also have decreased the aggregation tendency of the β-glucan molecules.

4. Conclusions

This study showed that acid or enzymatic hydrolysis at low water content enabled controlled depolymerisation of β-glucan in oat bran. However, the reaction mechanisms of acid- and enzyme-catalysed hydrolyses were rather different. Acid hydrolysed both (1 → 3)- and (1 → 4)-β-D-linkages, whereas the enzyme preparation seemed to cleave mainly (1 → 4)-β-D-linkages. In acid-hydrolysis, the degradation was controlled by temperature. A clear change in the M_w of β-glucan (from 86,000 to 34,000 g/mol) as well as in the subsequent dispersion stability occurred between 120 and 130 °C. In the enzymatic hydrolysis, the degradation was mainly dependent on the incubation time. The M_w of β-glucan was reduced from 71,000 to 49,000 g/mol between 3 and 4 h incubations with the enzyme preparation. When stored at 5 °C, the hot water extracts of acid-hydrolysed oat bran (M_w = 34,000 g/mol) were stable for 2 weeks at 1.8% and for 7 weeks at 1.4% β-glucan concentration, respectively. Similarly, the water extracts of enzyme-hydrolysed oat bran (M_w = 49,000 g/mol) were stable for 2 weeks at 1.9% and for 12 weeks at 1.6% β-glucan concentration. Enzyme-catalysed hydrolysis was superior to acid-hydrolysis in requiring less harsh conditions, not producing inorganic side streams, and resulting in more stable extracts. The stability of the acid- and enzyme-hydrolysed oat bran extracts could also be affected by other bran components, such as proteins, starch and arabinoxylans. However, the associations between β-glucan and other bran components were not subject to this study, and should be studied in future work.

Role of the funding source

This research was jointly funded by the Academy of Finland (a state-owned funding agency promoting scientific research in Finland), The Finnish Graduate School on Applied Bioscience: Bioengineering, Food & Nutrition, Environment (ABS) and VTT Technical Research Centre of Finland. The research was carried out at VTT, and the Academy of Finland and the ABS had no role in the research work or in the publishing of the results.

Acknowledgements

Funding from the Academy of Finland and the Finnish Graduate School on Applied Bioscience is gratefully acknowledged. Aliki Ninios, Mai Järvinen, Ulla Holopainen-Mantila, Arja Viljamaa, Leena Öhrnberg, Liisa Änäkäinen and Ritva Heinonen at VTT are acknowledged for their excellent assistance in chemical and microscopic analyses. The authors thank Michael Bailey for revising the English language.

References

AACC (2003). *Approved methods* (10th ed.). St. Paul, MN: American Association of Cereal Chemist.

Agbenorhevi, J. K., Kontogiorgos, V., Kirby, A. R., Morris, V. J., & Tosh, S. M. (2011). Rheological and microstructural investigation of oat β-glucan isolates varying in molecular weight. *International Journal of Biological Macromolecules*, *49*, 369–377.

Ajithkumar, A., Andersson, R., & Åman, P. (2005). Content and molecular weight of extractable β-glucan in American and Swedish oat samples. *Journal of Agricultural and Food Chemistry*, *53*, 1205–1209.

Åman, P., & Andersson, A. A. M. (2008). Oats full of soluble fibre. *Agro Food Industry Hi-Tech*, *19*, 25–27.

Åman, P., Rimsten, L., & Andersson, R. (2004). Molecular weight distribution of β-glucan in oat-based foods. *Cereal Chemistry*, *81*, 356–360.

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

Bailey, M., & Linko, M. (1990). Production of β-galactosidase by *Aspergillus oryzae* in submerged bioreactor cultivation. *Journal of Biotechnology*, *16*, 57–66.

Biörklund, M., van Rees, A., Mensink, R. P., & Önning, G. (2005). Changes in serum lipids and postprandial glucose and insulin concentrations after consumption of beverages with β-glucans from oats or barley. A randomised dose-controlled trial. *European Journal of Clinical Nutrition*, *59*, 1272–1281.

Blakeney, A. B., Harris, P. J., Henry, R. J., & Stone, B. A. (1983). A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydrate Research*, *113*, 291–299.

Böhm, N., & Kulicke, W. -M. (1999). Rheological studies of barley (1 → 3)(1 → 4)-β-glucan in concentrated solution: Investigation of the viscoelastic flow behaviour in the sol-state. *Carbohydrate Research*, *315*, 293–301.

Brown, L., Rosner, B., Willett, W. W., & Sacks, F. M. (1999). Cholesterol-lowering effects of dietary fiber: A meta-analysis. *American Journal of Clinical Nutrition*, *69*, 30–42.

Buliga, G. S., Brant, D. A., & Fincher, G. B. (1986). The sequence statistics and solution conformation of a barley (1 → 3)(1 → 4)-β-D-glucan. *Carbohydrate Research*, *157*, 139–156.

Cho, K. C., & White, P. J. (1993). Enzymatic analysis of β-glucan content in different oat genotypes. *Cereal Chemistry*, *70*, 539–542.

Doublier, J. -L., & Wood, P. J. (1995). Rheological properties of aqueous solutions of (1 → 3)(1 → 4)-β-D-glucan from oats (*Avena sativa* L.). *Cereal Chemistry*, *72*, 335–340.

Duss, R., & Nyberg, L. (2004). Oat soluble fibers (β-glucans) as a source for healthy snack and breakfast foods. *Cereal Foods World*, *49*, 320–325.

Flander, L., Salmenkallio-Marttila, M., Suortti, T., & Autio, K. (2007). Optimization of ingredients and baking process for improved wholemeal oat bread quality. *LWT-Food Science and Technology*, *40*, 860–870.

Forssell, P., Kontkanen, H., Schols, H., Hinz, S., Eijsink, V., Treimo, J., et al. (2009). Hydrolysis of brewer's spent grain by carbohydrate degrading enzymes. *Journal of the Institute of Brewing*, *114*, 306–314.

Hari Krishna, S. (2002). Developments and trends in enzyme catalysis in non-conventional media. *Biotechnology Advances*, *20*, 239–266.

Hobbs, H. R., & Thomas, N. R. (2007). Biocatalysis in supercritical fluids, in fluorous solvents, and under solvent-free conditions. *Chemical Reviews*, *107*, 2786–2820.

IUPAC, International Union of Pure and Applied Chemistry (1987). Measurement of cellulase activities. *Pure and Applied Chemistry*, *59*, 257–268.

Johansson, L., Virkki, L., Anttila, H., Esselström, H., Tuomainen, P., & Sontag-Strohm, T. (2006). Hydrolysis of β-glucan. *Food Chemistry*, *97*, 71–79.

Kaukovirta-Norja, A., Lehtinen, P., Virkajärvi, I., Suortti, M., Myllymäki, O., Helin, J., et al. (2009). *Method of processing β-glucan.* PCT application, WO 2009/077659 A1.

Kerckhoffs, D. A., Hornstra, G., & Mensink, R. P. (2003). Cholesterol-lowering effect of beta-glucan from oat bran in mildly hypercholesterolemic subjects may decrease when beta-glucan is incorporated into bread and cookies. *American Journal of Clinical Nutrition*, *78*, 221–227.

Kivelä, R., Gates, F., & Sontag-Strohm, T. (2009). Degradation of cereal β-glucan by ascorbic acid induced oxygen radicals. *Journal of Cereal Science*, *49*, 1–3.

Laakso, S., & Lehtinen, P. (2005). *Fiber composition in liquid form.* PCT application, WO 2006/040395 A1.

Lazaridou, A., & Biliaderis, C. G. (2004). Cryogelation of cereal β-glucans: Structure and molecular size effects. *Food Hydrocolloids*, *18*, 933–947.

Lazaridou, A., Biliaderis, C. G., & Izydorczyk, M. S. (2003). Molecular size effects on rheological properties of oat β-glucans in solution and gels. *Food Hydrocolloids*, *17*, 693–712.

Lazaridou, A., Biliaderis, C. G., Micha-Screttas, M., & Steele, B. R. (2004). A comparative study on structure–function relations of mixed-linkage (1 → 3), (1 → 4) linear β-D-glucans. *Food Hydrocoloids*, *18*, 837–855.

Lehtomäki, I., & Myllymäki, O. (2009). *Method of producing a bran product.* PCT application, WO 2009/109703 A1.

McCleary, B. V., & Matheson, N. (1987). Enzymic analysis of polysaccharide structure. *Advances in Carbohydrate Chemistry and Biochemistry*, *44*, 147–276.

Moore, J., Cheng, Z., Su, L., & Yu, L. (2006). Effects of solid-state enzymatic treatments on the antioxidant properties of wheat bran. *Journal of Agricultural and Food Chemistry*, *54*, 9032–9045.

Naumann, E., van Rees, A. B., Önning, G., Öste, R., Wydra, M., & Mensink, R. P. (2006). β-Glucan incorporated into a fruit drink effectively lowers serum LDL-cholesterol concentrations. *American Journal of Clinical Nutrition*, *83*, 601–605.

Othman, R. A., Moghadasian, M. H., & Jones, P. J. H. (2011). Cholesterol-lowering effects of oat β-glucan. *Nutrition Reviews*, *69*, 299–309.

Poutanen, K., Rättö, M., Puls, J., & Viikari, L. (1987). Evaluation of different microbial xylanolytic systems. *Journal of Biotechnology*, *6*, 49–60.

Reinikainen, T., Henriksson, K., Siika-aho, M., Teleman, O., & Poutanen, K. (1995). Low-level endoglucanase contamination in a *Trichoderma reesei* cellobiohydrolase II preparation affects its enzymatic activity on β-glucan. *Enzyme and Microbial Technology*, *17*, 888–892.

Ren, Y., Ellis, P. R., Ross-Murphy, S. B., Wang, Q., & Wood, P. J. (2003). Dilute and semi-dilute solution properties of (1 → 3), (1 → 4)-β-D-glucan, the endosperm cell wall polysaccharide of oats (*Avena sativa* L.). *Carbohydrate Polymers*, *53*, 401–408.

Ripsin, M. C., Keenan, J. R., Jacobs, D. R., Elmer, P. J., Welch, P. R., Van Horn, L., et al. (1992). Oat products and lipid lowering. *JAMA: The Journal of the American Medical Association*, *267*, 3317–3325.

Saastamoinen, M., Plaami, S., & Kumpulainen, J. (1992). Genetic and environmental variation in β-glucan content of oats cultivated or tested in Finland. *Journal of Cereal Science*, *16*, 279–290.

Santala, O., Lehtinen, P., Nordlund, E., Suortti, T., & Poutanen, K. (2011). Impact of water content on the solubilisation of arabinoxylan during treatment of wheat bran. *Journal of Cereal Science*, *54*, 187–194.

Santala, O. K., Nordlund, E. A., & Poutanen, K. S. (2012). Treatments with xylanase at high (90%) and low (40%) water content have different impacts on physicochemical properties of wheat bran. *Food and Bioprocess Technology*. http://dx.doi.org/10.1007/s11947-012-0967-6.

Sibakov, J., Myllymäki, O., Holopainen, U., Kaukovirta-Norja, A., Hietaniemi, V., Pihlava, J. -M., et al. (2011). Lipid removal enhances separation of oat grain cell wall material from starch and protein. *Journal of Cereal Science*, *54*, 104–109.

Sikora, P., Tosh, S. M., Brummer, Y., & Olsson, O. (2013). Identification of high β-glucan oat lines and localization and chemical characterization of their seed kernel β-glucans. *Food Chemistry*, *137*, 83–91.

Suortti, T. (1993). Size-exclusion chromatographic determination of β-glucan with post-column reaction detection. *Journal of Chromatography*, *632*, 105–110.

Tosh, S. M., Brummer, Y., Miller, S. S., Regand, A., Defelice, C., Duss, R., et al. (2010). Processing affects the physicochemical properties of β-glucan in oat bran cereal. *Journal of Agricultural and Food Chemistry*, *58*, 7723–7730.

Tosh, S. M., Wood, P. J., Wang, Q., & Weisz, J. (2004). Structural characteristics and rheological properties of partially hydrolyzed oat β-glucan: The effects of molecular weight and hydrolysis method. *Carbohydrate Polymers*, *55*, 425–436.

Vaikousi, H., Biliaderis, C. G., & Izydorczyk, M. S. (2004). Solution flow behavior and gelling properties of water-soluble barley (1 → 3,1 → 4)-β-glucans varying in molecular size. *Journal of Cereal Science*, *39*, 119–137.

Vårum, K. M., Smidsrød, O., & Brant, D. A. (1992). Light scattering reveals micelle-like aggregation in the (1 → 3), (1 → 4)-β-D-glucans from oat aleurone. *Food Hydrocolloids*, *5*, 497–511.

Wolever, T. M. S., Tosh, S. M., Gibbs, A. L., Brand-Miller, J., Duncan, A. M., Hart, V., et al. (2010). Physicochemical properties of oat β-glucan influence its ability to reduce serum LDL cholesterol in humans: A randomized clinical trial. *American Journal of Clinical Nutrition*, *92*, 723–732.

Wu, J., Zhang, Y., Wang, L., Xie, B., Wang, H., & Deng, S. (2006). Visualization of single and aggregated hulless oat (*Avena nuda* L.) (1 → 3),(1 → 4)-β-D-glucan molecules by atomic force microscopy and confocal scanning laser microscopy. *Journal of Agricultural and Food Chemistry*, *54*, 925–934.

Zhang, M., Bai, X., & Zhang, Z. (2011). Extrusion process improves the functionality of soluble dietary fiber in oat bran. *Journal of Cereal Science*, *54*, 98–103.

Zheng, G. -H., Hess, R., Khare, A., Hilbert, B., & Deguise, M. (2004). *Improved dietary fiber containing materials comprising low molecular weight glucan.* PCT application, WO 2004/086878 A2.

Zurbriggen, B. Z., Bailey, M. J., Penttilä, M. E., Poutanen, K., & Linko, M. (1990). Pilot scale production of a heterologous *Trichoderma reesei* cellulase in *Saccharomyces cerevisiae*. *Journal of Biotechnology*, *13*, 267–278.