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Oxidative and radical mediated cleavage of β-glucan in thermal treatments

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ABSTRACT

Oat β-glucan is a dietary fibre with solid and well evidenced health benefits. Its beneficial properties, however, largely depend on its molecular properties and processing history. In this study, the cleavage of oat β-glucan in heat treatments (95 °C and 120 °C) and role of oxidative reactions were investigated in highly purified β-glucan solution and in β-glucan extracts of oat bran. Both viscosity and molar mass decreased during the heat-treatments, and the degradation was more intensive in the extract solutions with co-extracted phytates, proteins, minerals and other compounds. Oxidative reactions, most likely Fenton chemistry, played a role in the degradation, since oxidative free radicals and hydrogen peroxide were formed during the heating, and the addition of an oxidation catalyst (Fe^{2+}-ions) accelerated the thermal degradation. Inhibition of the oxidative reactions by antioxidants appeared to be challenging; for example, ascorbic acid alone accelerated the thermal degradation of β-glucan, but its use with sulfite slowed down the rate of thermal degradation.

1. Introduction

The most abundant soluble fibre of oat, (1 → 4),(1 → 3)-β-D-glucan (referred here to as β-glucan), is a cereal cell wall polysaccharide, which has attracted attention with its beneficial effects on human health. Chemically cereal β-glucan is a linear uncharged glucose chain, where the monomers are linked by 1 → 4-β-linkages and 1 → 3-β-linkages (Wood, 1993). The 1 → 3-β-linkages interrupt cellulose-like regions formed by conjunctive 1 → 4-β-linkages, and enable water solubility of β-glucan. The water solubility is a prerequisite for its health effects and technological properties. Native β-glucan ($M_w = 1000–3000 \times 10^3$ g/mol) forms highly viscous water solutions, which is often related to the health promoting effects (Wood, 2010). Thus, any changes in its solution properties and molecular properties are of crucial interest and, thus, the degradation mechanisms during processing are important to understand.

Heating influences polysaccharides by accelerating the molecular vibration, their collisions and chemical reactions in solutions. All these may lead to fragmentation or depolymerisation of polysaccharide chain (Pielichowski & Njuguna, 2005, chapter 2). The rate and the type of products formed depend on the degradation mechanisms, which are altered by temperature, heating time and polysaccharide molecular property as well as the solution type. The heat induced cleavage of glycosidic bonds is catalysed by OH^--ions and H_3O^+-ions, which makes thermal degradation strongly pH-dependent. Solvent quality and polysaccharide concentration affect the rate of thermal degradation as well as charge, molar mass, structure and shape of the polysaccharide (Soldi, 2005, chapter 14). For example, linear carboxymethylcellulose and carrageenan showed a relatively good thermal stability compared to branched alginate, xanthan and different galactomannans studied as viscosity changes (Bradley & Mitchell, 1988; Mitchell, Reed, Hill & Rogers, 1991). Cereal β-glucan degrades in elevated temperatures in acidic and alkali conditions (Beer Wood, Weisz & Fillion, 1997; Johansson et al., 2006; Temelli, 1997; Vaikousi & Biliaderis, 2005). In pure and neutral aqueous solution, oat β-glucan cleaved at the conditions of autoclave sterilization, whereas only the degree of aggregation of other neutral polysaccharides xyloglucan and dextran was affected (Wang, Wood & Ross-Morphy, 2001).

Oxygen and transition metals accelerate thermal degradation of the polysaccharides and are critical for its initiation. For example, the rate of thermal combustion of cellulose and corn starch increased to 5 and 9-fold, respectively, as the nitrogen was replaced by oxygen atmosphere (Aggarwal, Dollimore & Heon, 1997). Thermal oxidation initiates when alkyl and hydroperoxyl radicals are formed in the reactions of molecular oxygen and organic compounds, or in the thermal decomposition of organic compounds (Robert, Barbati, Ricq & Ambrosio, 2002). The propagation reactions produce strong oxidants, including reactive oxygen species such as hydroxyl radicals and peroxides. Within the free radical formation, the occurrence of hydrogen peroxide is of particular interest, since it can produce hydroxyl radicals in transition metal catalysed reaction, often called Fenton reaction ((reaction (1), Halliwell

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& Gutteridge, 1984). The Fenton reaction is part of Haber–Weiss process, which is one of the most frequently cited mechanism for macromolecular oxidation in biological conditions (Barbusinski, 2009).

$$Cu^{+}/Fe^{2+} + H_2O_2 + H^{+} \rightarrow Cu^{2+}/Fe^{3+} + H_2O + {}^{\bullet}OH \text{ Fenton} \quad (1)$$

$$Cu^{2+}/Fe^{3+} + RO^{\bullet} + H_2O \quad (2)$$

Hydroxyl radicals can attack to pccharide backbone, abstract a hydrogen atom and thus enable oxygen attack to the chain. This results in a stable and/or labile carbonyl formation, of which the latter leads to the cleavage of the chain (von Sonntag, 1980). Cereal β-glucan suffered oxidative cleavage at temperatures of 5–25 °C (Kivelä, Gates & Sontag-Strohm, 2009; Kivelä, Nyström Salovaara & Sontag-Strohm, 2009), but the role of elevated temperatures in the oxidation of cereal β-glucan has not been previously studied.

The aim of this study was to investigate mechanisms of thermal degradation of β-glucan. The studies were carried out with β-glucan extracts and solution of highly purified β-glucan in purpose to investigate the role of co-extracted impurities present in foods and analysis. Contribution of oxidation reactions in thermal degradation of β-glucan was studied as the formation of the reactive oxygen species and consumption of oxygen. For the management aspect, inhibition effect of antioxidants was studied.

2. Materials and methods

2.1. Preparation and characterisation of the β-glucan solutions

Two oat brans, Oat Bran Concentrate supplied by Raisio Oyj (Raisio, Finland) and OatWell14% supplied by Swedish Oat Fibre (Våröbacka, Sweden), were studied. Solution extracted from the Oat Bran Concentrate was named OBC and solution from the OatWell14% was named OBCet, since it was an extract of the Oat Bran Concentrate manufactured using ethanol treatment. Oat brans were extracted with MilliQ water (Millipore system, Lab water, USA) at 40 °C for 30 min in a shaking incubator. After centrifuging the dispersions for 10 min with 16,000 × g, supernatant was collected and placed in a boiling water bath for 10 min to precipitate proteins. After boiling, the dispersions were centrifuged again and the supernatant was used as the sample solution. OatWell14% was weighed in ratio 6 g bran per 100 ml water and Oat Bran Concentrate in ratio of 4 g per 100 ml.

Medium viscosity oat (1 → 3),(1 → 4)-β-D-glucan (>99%,) (MVO) was supplied by Megazyme International Ltd. Stock solution MVO was prepared by wetting the powder with 99% ethanol (Altia, Finland) before adding the MilliQ water to dissolve the β-glucan. The dispersion was heated 3 h at 80 °C until a clear solution was obtained.

Quantification of β-glucan in the extracts was performed with an enzymatic method (Approved Method 32-23, AACC 2000) using a Megazyme kit BBG (Megazyme, International Ltd, Ireland). Moisture content of the extracts was measured according to Approved Method 44-60 (AACC 2000) with minor modifications (Degutyte-Fomins, Sontag-Strohm & Salovaara, 2002). The quantification was performed in triplicate from the oat bran extracts of all series prior to sample preparation.

Composition and quantity of monosaccharides (glucose, xylose, mannose, arabinose, fructose and galactose) and the quantity of total carbohydrates were analysed with gradient high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD system) equipped with two HPLC-pumps, an autosampler, and an electrochemical detector, and Empower 2 software for data handling (Waters corporation, USA). The analytical CarboPac PA-1 column with the guard column (Dionex Corporation, USA) was maintained at, 30 °C. The gradient analysis used eluents H_2O and 0.2M NaOH with total eluent flow rate of 1 ml/min. In prior to the analysis, the carbohydrates of extracts were acid hydrolysed with 4% sulfuric acid into monosaccharides in 1 h autoclaving at 120 °C according to the methods of NREL LAP (*2nd step of mild acid hydrolysis*, Sluiter et al., 2008). Hydrolysis was done in triplicates and in prior to the analysis the monosaccharide solutions were neutralized with NaOH to a pH range of 5–7. A correction factor (1.1–1.3) was used based on the loss of pure monosaccharides during the acid hydrolysis.

Protein content of the solutions was analysed from lyophilized extracts and from the powder of pure β-glucan as triplicate using the Dumas combustion method (Vario MAX CN Elementar Analyser systeme GmbH, Germany) and nitrogen factor of 6.25.

Mineral content was analysed from lyophilized extracts and from the powder of pure β-glucan after wet-digestion (HNO_3) using inductively coupled plasma-mass spectrometer (ICP-MS, Perkin-Elmer Elan 6000, USA) with external standard method.

Fatty acid content was determined from OBC and OBCet by extracting lipids with acidic diethyl ether with added internal standard (methyl nonadecanoate, 5.0 mg, 99%). Fatty acids were methylated with boron trifluoride before analysis by capillary gas chromatography (Laiho et al., 2003).

2.2. Heat-treatments of β-glucan solutions

The β-glucan solutions MVO, OBC and OBCet were treated with iron(II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$, E. Merck, Germany) solution so that the concentration of the sample solutions resulted in 50 μM of $FeSO_4 \cdot 7H_2O$. L(+)-Ascorbic acid (J.T. Baker, The Netherlands) and/or potassium sulfite (K_2SO_3, Sigma–Aldrich Chemie GmbH, Germany) were added to the β-glucan solutions as powders. The samples were heated at 95 °C in a dry incubator or at 120 °C in an autoclave in 15 ml tubes with 5 ml sample for given times. The caps were as tight as possible, and the evaporation was ensured not to occur by weighting the tubes + samples before and after the heat-treatments. The temperature was controlled with an internal sensor. After heating, the samples were let to settle down at +6 °C prior to viscometric, spectrophotometric and chromatographic analyses.

2.3. Analysis of thermal degradation and occurrence of oxidative reactions

Viscosity of the solutions was measured by ThermoHaake RheoStress 600 rheometer (Thermo Electron GmbH, Germany). The viscosity was measured from the samples treated 30 min at 95 °C and 5 min at 120 °C with and without additives. Control samples (untreated) were stored at 6 °C, prior to the measurements. The solutions were stabilised at +6 °C for 15 min and 3 h before the viscosity measurements, and the measurements were repeated after 24 h and 48 h to assure the irreversible effect of the treatments. The results at 3 h were repeatable unlike the samples after 15 min stabilising time, and thus the viscosity results at time point 3 h of cooling were recorded and compared to the viscosity of untreated β-glucan solutions. A flow curve was obtained using a cone and plate geometry (60 mm, 1°) over a shear rate range of 0.3–300–0.3 s^{-1}. All the rheological experiments were performed at +10 °C. The equipment was controlled with standard oils during the study and the method-related variation was ±15 mPa s.

Polymeric parameters (M_w, R_g, R_h and α) were analysed from the heated heat-treated (95 °C and 120 °C) β-glucan solutions with and without ferrous ion addition by a high performance size exclusion chromatography (HPLC)-system equipped with light scattering (λ_0 = 670 nm), viscometric (270 Dual Detector, Viscotek Corp.) and a refractive index (RI) detectors (VE 3580, Viscotek Corp.). Two linear type Shodex columns (OHpak SB-806M HQ, 8 mm × 300 mm, exclusion limit 2×10^7, Showa Denko) were used with a guard column (OHpak SB-6, 4.6 mm × 10 mm) and an aqueous eluent (0.1 M $NaNO_3$) as previously reported (Kivelä, Pitkänen, Laine, Aseyev & Sontag-Strohm, 2010). The molar masses were calculated based on the light scattering/viscometry method using the dn/dc value of 0.151 ml/g (Gomez, Navarro, Manzanare, Horta & Carbonell, 1997). Weight average molar mass (M_w), radius of gyration (R_g), hydrodynamic radius (R_h) and Mark–Houwink's parameter (α) were analysed within 2–3 h after the heat-treatments.

Formation of free radicals was monitored with an electron spin resonance (ESR) – spectroscopy method using POBN [α-4-pyridyl(1-oxide)-N-tert-butylnitrone, Aldrich, St. Louis, MO] spin trapping. Sample solutions were mixed with POBN-solution in ratios which resulted in concentration of 40 mM of POBN with stirring 10 min at room temperature. Ethanol was added to oat bran extracts (4%) to increase the sensitivity/selectivity of detection, but in the case of MVO no additional ethanol than the one added in the solubilisation step was needed. The sample solutions were set into a preheated water bath (95 °C) in tightly closed eppendorf tubes (2 ml) with a 0.6 ml sample, and the sample aliquots were collected at given time intervals. ESR spectra were recorded with a Miniscope MS 300 X-band spectrometer using 50 μl micropipettes as sample cells (Brand GMBH, Wertheim, Germany). The ESR settings were: microwave power, 10 mW; sweep width, 95.9 G; modulation frequency, 1000 mG; receiver gain, 900; and sweep time, 60 s. All spectra, consisting of four scans, were recorded at room temperature. The amplitudes of the spectra were measured and reported as the height of the central doublet relative to the height of the central line in the ESR signal of an aqueous TEMPO solution (2 μM). The TEMPO (stable radical reference, 2,2,6,6-Tetramethylpiperidine-1-oxyl, 99%, Sigma–Aldrich) was used as a reference sample. All samples were measured in triplicate. The free radical formation was studied from all the solutions with and without the additives at temperatures 95 °C and 21 °C. The 120 °C was not included due to the instability of the spin trap.

Hydrogen peroxide content of the heat-treated (95 °C and 120 °C) β-glucan solutions without ferrous ion addition was analysed by a ferrous oxidation xylenol orange method (FOX) in given time intervals. The acidic FOX reagent containing excess amount of reduced iron (Fe^{2+}) was prepared daily by mixing aqueous xylenol orange and D-sorbitol (0.137 mM and 0.1 M, respectively) with a stock solution of $FeSO_4$, $(NH_4)_2SO_4$ and H_2SO_4 (25 mM, 25 mM and 2.5 M). Sample (100 μl) was incubated for 30 min at room temperature with FOX (1 ml) reagent, and the absorbance (A1) of xylenol orange-oxidized iron (Fe^{3+})-complex was measured at 560 nm (Perkin Elmer, Waltham, USA). The content of hydrogen peroxide was calculated against an external standard curve (2–00 μg/l of H_2O_2) and the effect of matrix was eliminated by subtracting the absorbance of acidic matrix at 560 nm from the A1. Catalase was added, after the heat-treatments in the concentration of 6000 U/l of sample, before FOX analysis to investigate whether the measured H_2O_2-equivalents were actually hydrogen peroxide or other oxidizing compounds.

Oxygen consumption was estimated by measuring the amount of dissolved oxygen of the solutions with and without ferrous ion addition during the heating at 95 °C and 120 °C by a Mettler-Toledo MO128 oxygen meter. Prior to the measurements, the meter was calibrated to 100% (saturation degree) in a headspace of a flask containing MilliQ water in the bottom and air at room temperature (21 °C). The samples were heated in closed vials (15 ml) with sample amount of 11 ml. Before the measurements, the samples were carefully cooled to room temperature (21 °C) in a water bath with controlled water temperature and sealed caps (21 °C). When measuring, the caps were opened, the meter settled to the solution and vials without headspace were resealed with parafilm. The oxygen concentration was measured as mg/l and presented here also as mM.

Table 1
The content of β-glucan, carbohydrates, proteins, fatty acids and selected minerals in the studied β-glucan solutions, dw is the weight of the dry matter of the solutions.

	MVO	OBC	OBCet
β-Glucan (mg/ml)[a]	9.9	1.2 ± 0.2	1.5 ± 0.2
β-Glucan (%, w/dw)[a]	99	35 ± 4	40 ± 4
Carbohydrates (%, w/dw)[b]	n.d.	57 ± 1	58 ± 2
Glucose (% of total)	n.d.	89	86
Galactose (% of total)	n.d.	5	6
Arabinose (% of total)	n.d.	2	3
Xylose (% of total)	n.d.	2	3
Proteins (%, w/dw)	<1	10 ± 1	15 ± 1
Fatty acids (%, w/dw)	n.d.	0.06	0.03
16:0 (% of total)	n.d.	20	20
18:0 (% of total)	n.d.	4	71
18:1 (% of total)	n.d.	39	0
18:2 (% of total)	n.d.	37	0
Minerals (mg/100 g dw)			
Fe	13.0	3.5	2.5
Cu	0.3	1.3	2.4
Zn	3.0	4.0	4.5
P	390	1250	1900
Dry weight (dw, %)	1	0.34 ± 0.07	0.38 ± 0.08
pH	6.9	6.4	6.6

[a] Enzymatic analysis.
[b] HPLC-analysis after acid hydrolysis.

2.4. Statistical analysis

A non-parametric correlation (Spearman and Kendall rank correlation coefficients, r_{ho} and r_{K}, respectively) was used to the determination of the relation of viscosity decrease and oxidation reactions, since the dipolar nature of the parametric data. The slopes of the exponential phase of the radical formation data at 95 °C for 30 min ((relative radical formation/t) – b, where t is time and b is a coefficient of the linear function) were used as a pair of change in the molar mass $[1-(M_{w,\mathrm{treated}}/M_{w,\mathrm{original}})]$ and change in viscosity $[1-(\eta_{\mathrm{treated}}/\eta_{\mathrm{original}})]$ after 30 min heating at 95 °C. To study the significance of the differences within sample series of one parameter, analysis of variance and multiple range tests were applied. A confidence level of 95% was required for the differentiation of samples. The analyses were performed with SPSS 4.0-software.

3. Results and discussion

3.1. Characterisation of the solution compositions

Of the three solutions studied, two represented food-like conditions (OBC and OBCet extracts) containing several co-extracting components. Extracts contained 35–40% of β-glucan in their dry matter, and the study solutions contained 1.0–1.7 mg/ml β-glucan (Table 1). The total amount of carbohydrates was 56–60% of the dry weight, of which over 85% was glucose, and the rest consisted of arabinose, xylose and galactose (Table 1). The higher glucose content obtained by the HPLC-method (≈50%) than obtained by the enzymatic method (≈40%) is partially explained by the oligosaccharides with DP < 10, which are not included in the enzymatic method. The extraction ratio was chosen to achieve similar viscosities between the extracts (120–170 mPa s). The third solution was a highly purified commercial β-glucan (MVO, purity >99%), which was studied at concentration of 10 mg/ml (η_{app} (10 s^{-1}) = 50–60 mPa s).

The amount of endogenous transition metals, iron, copper and zinc was analysed, since they are common catalysts in oxidation reactions (Vreeburg & Fry, 2005). Phosphorous was also analysed,

Table 2
Effect of heating (120 °C for 5 min and 95 °C for 30 min) on the viscosity, molar mass and conformation-related Mark–Houwink's α-value in the β-glucan solutions.

	No heating		95 °C 30 min		120 °C 5 min	
	Plain	+Fe^{2+}	Plain	+Fe^{2+}	Plain	+Fe^{2+}
η_{app} (MVO, mPa s)	51 ± 2	49 ± 2	47 ± 2	41 ± 2	45 ± 2	34 ± 4
$\eta_{app,treated}/\eta_{app,untr.}$ (%)	100	96	92	80*	89*	67*
M_w (MVO, $\times 10^3$ g/mol)	284	286	271	260	254	241
α (MVO)	0.63	0.63	0.60	0.59	0.65	0.65
η (OBC, mPa s)	114 ± 5	49 ± 18	100 ± 14	20 ± 3	7 ± 3	2 ± 1
$\eta_{app,treated}/\eta_{app,untr.}$ (%)	100	17*	88	18*	11*	4*
M_w (OBC, $\times 10^3$ g/mol)	2300	1809	1480	1095	1546	1119
η (OBCet, mPa s)	159 ± 17	40 ± 14	141 ± 13	45 ± 13	39 ± 7	7 ± 0.5
$\eta_{app,treated}/\eta_{app,untr.}$ (%)	100	24*	93	23*	21*	3*
M_w (OBCet, $\times 10^3$ g/mol)	1300	1068	1202	932	975	543
α (OBCet)	0.77	0.74	0.76	0.72	0.71	0.69

* A statistically significant difference in the viscosity from the untreated sample with at least 95% confidence level.

as it is associated with the endogenous chelator, phytic acid (myo-inositol phosphate, Maga, 1982). The amount of iron and zinc in both extracts was in range 2.5–4.5 mg/100 g of the dry weight (Table 1). Content of iron was approximately four times higher in the purified β-glucan powder than in the dry matter of the oat bran extracts. Consistently, the amount of proteins, lipids and phosphorous was lower in the highly purified β-glucan powder than in the dry matter of extracts. The abundance of iron in higher concentrations in purified β-glucan sample extract suggests that iron was rather associated with β-glucan than with phytate or proteins. Majority of the added $FeSO_4 \cdot 7H_2O$ has been reported to complex with barley β-glucan solution at pH 6.5 (Platt & Clydesdale, 1984). In contrast to the iron, the content of copper was lower in the pure β-glucan (MVO powder) than in the dry matter of the extracts.

3.2. *Thermal degradation of β-glucan*

The solution properties of oat β-glucan are fundamental to its technological and physiological functionality and, thus, the study was based on viscosity changes in heating processes (95 °C and 120 °C). Both heat-treatments affected the viscosity and molecular behavior of β-glucan (Table 2). The treatment of 120 °C for 5 min resulted in decreased viscosity of the solutions and decreased molar mass of β-glucan. The power law correlation between the viscosity and molar mass (Fig. 1), especially in the case of MVO, was similar to the previous studies of hydrolysed β-glucan (Tosh, Wood, Wang & Weisz, 2004). This indicates that viscosity decrease was mainly due to backbone cleavage of β-glucan and not to conformational changes of the molecule. Conformation-related Mark–Houwink's α-values remained in the range of the typical value for random coils (0.60–0.77), widely reported for oat β-glucan as well, during the heat treatments (Table 2). Not only 120 °C, but also 95 °C caused a decrease in the viscosity of the solutions and molar mass of β-glucan (Table 2).

In the unpurified extracts OBC and OBCet, the autoclaving treatment caused a drastic loss in viscosity unlike in the solutions of pure β-glucan (Table 2). After autoclaving, viscosity decreased to 10% and to 20% of the original viscosity of OBC and OBCet, whereas the viscosity of purified β-glucan solutions was approximately 90% of their original viscosity. This can be hypothesized to be due to the different compositions of solutions, and the minor constituents of the extracts, which may initiate chemical degradation such as oxidation.

The molar masses were analysed as the heating at 120 °C was continued up to 30 min. At time points 5, 15 and 30 min, the molar mass distributions of β-glucan linearly shifted towards lower molar mass species in MVO. In the extracts, the autoclaving treatments with the extended exposure time resulted in several mass populations and the weight average molar mass of the β-glucan did not decrease linearly with time as shown in Fig. 2. β-Glucan may radicalise or oxidize during the longer exposure times, which may lead to intermolecular cross-linking as shown by dry pullulan when heated at 160 °C (Strlic, Kocar, Kolar, Rychly & Pihlar, 2003). The degradation of pullulan was reported to be non-random at 160 °C, but random in 80 °C. Guar gum in a water solution resulted in fragmentation and depolymerisation to low molar mass species increasing the polydispersity when treated by 95–120 °C (Bradley, Ball, Harding & Mitchell, 1989). The reassociation and non-random scission may result in ambiguous values for the apparent weight average molar masses, although the exposure temperature and exposure time dependent thermal degradation of β-glucan was evident.

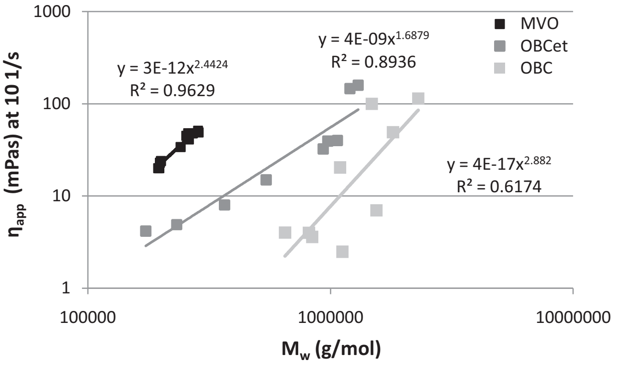

Fig. 1. A correlation plot of weight average molar mass (M_w) of β-glucan and the corresponding apparent viscosity (η_{app}) of MVO, OBC and OBCet before and after the heat-treatments at 95 °C for 30 min and at 120 °C for 5 min with and without ferrous ions.

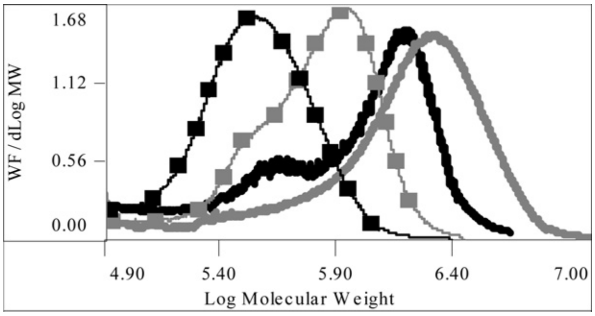

Fig. 2. Molar mass distributions of β-glucan in OBCet-samples after 5 min (gray line), 30 min (black line) and 15 min (gray line with solid rectangles) exposure time at 120 °C. The black line with solid rectangles is for OBCet sample which contained added Fe^{2+}-ions and was heated 30 min at 120 °C.

3.2.1. Role of aggregation in the viscosity decrease

The OBC solution had the weakest power law-correlation between the weight average molar mass and viscosity (Fig. 1). For instance, the M_w of β-glucan in autoclaved OBC was approximately 1500×10^3 g/mol, but had a water-like viscosity (η_{app} ($10\,s^{-1}$) = 7 mPa s), while similar molar mass of untreated OBCet gave η_{app} ($10\,s^{-1}$) ≈ 150 mPa s at consistent concentration. The low angle laser light scattering detected two populations of β-glucan as a split peak in the OBC solution, while in other solutions (OBCet and MVO) only one sharp population was detected. The heat-treatments mostly affected the population of higher molar mass (size) β-glucan in OBC, and only a slight shift towards lower molar mass species was observed. After treating at 120 °C, the higher molar mass population disappeared, but the concentration of the lower molar mass species did not practically increase. This may indicate aggregate dissociation by the heat-treatments, and also lower solubility of the low molar mass β-glucan or its complexes in this unpurified solution. The α-values of most OBC samples were 1.15–1.23 suggesting an aggregated structure and not valid estimation of the shape and mass of the molecule (Picout & Ross-Murphy, 2007).

In OBCet and in the pure solutions, the shape and size parameters suggested a relatively low aggregation degree of β-glucan in the time of molar mass analysis. Eventhough only one population was detected, the aggregation behavior of β-glucan is of crucial interest in aqueous environment, because in water solutions β-glucan occurs as relatively stiff aggregates and the aggregation is rapid (Grimm, Kruger & Burchard, 1995; Li, Cui, Wang & Yada, 2010). Hydrodynamic radius (R_h) for β-glucan in OBCet was measured to be 47 nm (M_w 1200×10^3 g/mol, R_g 71 nm) and in MVO 22 nm (M_w 280×10^3 g/mol, R_g 35 nm), which agree with the hydrodynamic radius of unimers reported for oat β-glucan by Li et al. (2010). They found R_hs 46 nm and 18 nm for unimers of oat β-glucan fractions with $M_{w,water}$ 2000×10^3 g/mol and 360×10^3 g/mol. The unimers were determined by light scattering methods in a cadoxen solution. Shape of the molecule can be determined by a parameter $\rho = R_g/R_h$, which depends on the chain architechture, conformation and polydispersity, but not on the molar mass (Burchard, 2005, chapter 7). For polydisperse random coils, $\rho = 2.05$ in a good solvent and $\rho = 1.73$ in theta-solvent, for star-branched structures $\rho \approx 1$ and for a rigid sphere $\rho \approx 0.7$ (Burchard, 2005; Grimm et al., 1995). In the present study the ρ-values were 1.5–1.7 for β-glucan in OBCet and MVO, which are consistent with the value reported for a wheat β-glucan unimer ($\rho = 1.7$; Li, Wang, Cui, Huang & Kakuda, 2006). For a barley β-glucan unimer, $\rho \approx 2.2$ has been reported and $\rho \approx 0.9$ for its aggregate in water solution (10 mg/ml) (Grimm et al., 1995). Unlike in OBC, in OBCet and in MVO, the β-glucan molecule appeared to aggregate in relatively low degree at the moment of HPSEC-analysis, and the thermal treatment-induced molecular degradation instead of dissociation of β-glucan is suggested in these solutions.

3.3. Contribution of oxidation reactions to the thermal degradation of β-glucan

3.3.1. Effect of added ferrous ions

Effect of heating on the solution properties was studied not only in different solutions, but also in the presence of oxidation related additive, Fe^{2+}-ions. The generally referred pro-oxidants were added from freshly prepared solutions in small amounts (50 μM), being just under 1% of the endogenous iron of MVO solutions and 2–3% of the endogenous (bound) iron of OBC and OBCet extracts. The iron addition accelerated the loss in viscosity and in molar mass at both temperatures in all solutions (Table 2), showing that the thermal degradation accelerated in the presence of the metallic catalyst. As we have previously reported (Kivelä, Nyström et al., 2009), the addition of iron elicited a decrease in the extract viscosity already without heating, whereas no decrease in the viscosity occurred in the pure solutions without additional energy from heating (Table 2).

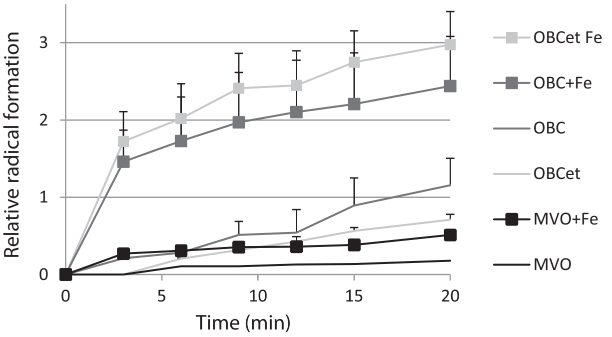

Fig. 3. The relative radical formation (sample signal intensity in relation to stable radical TEMPO) in the MVO, OBC and OBCet solutions with and without ferrous ions at 95 °C.

3.3.2. Formation of free radicals

Heating at 95 °C induced formation of the free radicals in each of the β-glucan solutions as shown as a relative radical formation in Fig. 3. No radicals were formed at room temperature, as the measurements were continued for 1.5 h. The formation was moderate in all solutions, but most intensive in the unpurified extract solutions in order of OBC > OBCet > MVO. When Fe^{2+}-ions were added, the radical formation accelerated, and was clear and signifigant in the extract solutions (Fig. 3). This agrees with previous results, which showed that the rate of radical formation increased by the addition of $FeSO_4$ in beer and wort (Frederiksen, Festersen & Andersen, 2008; Uchida & Ono, 1996). Based on the used method and previously reported results (Robert et al., 2002), the detected free radicals were assumed to be mostly strong oxidants, most likely hydroxyl ($^{\bullet}OH$) radicals. When ethanol is used in the sample solutions, the intensity and selectivity of the method for highly reactive strong oxidants are enhanced. The intensity is enhanced, since ethanol addition stabilises the signals by reacting rapidly with the highly reactive radicals, thus preventing them to react unselectively with carbohydrates and produce wide range of polysaccharide degradation products (Frederiksen et al., 2008). The selection of the reactive oxygen species among other free radicals is enhanced by the ethanol addition, since the oxidation of an alcohol requires a strong oxidant with a reduction potential at least 1200 mV ($E(OH^*) = 2300$ mV, Welch Davis & Aust, 2002).

3.3.3. Formation of hydrogen peroxide

Formation of hydrogen peroxide (H_2O_2) during the treatments at 95 °C and 120 °C was determined to achieve supportal data of the oxidizing potential and mechanisms. H_2O_2-equivalents increased significantly in the extract solutions and insignificantly in MVO when heated at 120 °C (Fig. 4). At 95 °C, a slight increase of the H_2O_2-equivalents in the extract solutions was obtained. When catalase, which effectively decomposes hydrogen peroxide, was added after the treatments in the solutions, the heat-induced increase in H_2O_2-equivalents was inhibited. This indicates that the H_2O_2-equivalents indeed measured hydrogen peroxide formation during the heat-treatments rather than other peroxides or more complex iron oxidation. Hydrogen peroxide formation is generally reported as a result of intensive heating of polysaccharides, but the origin of hydrogen peroxide has often remained uncertain (Robert et al., 2002). For instance, the radical formation in heated cellulose solutions was dependent on hydrogen peroxide (Robert et al., 2002). Consistently, hydrogen peroxide participated the radical formation

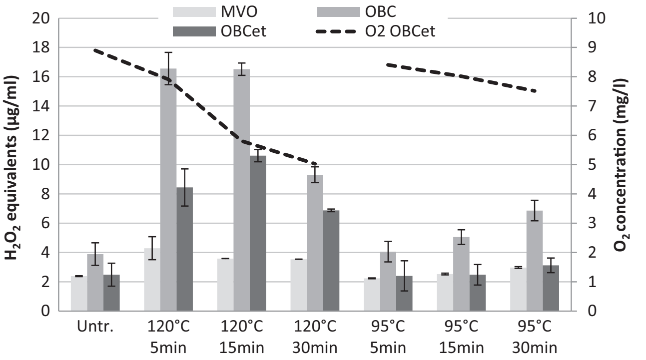

Fig. 4. Hydrogen peroxide formation in MVO, OBC and OBCet and concentration of dissolved oxygen after treatments at 95 °C and 120 °C for 5 min, 15 min and 30 min.

in wort, but could not however explain it entirely (Frederiksen et al., 2008). Qian and Buettner (1999) reported only a slight inhibition of free radical formation by catalase alone, but together with superoxide dismutase, which effectively decomposes the superoxide, the inhibition was significant.

3.3.4. *Oxygen consumption*

Oxygen was consumed in all the solutions in significantly higher rates at 120 °C than at 95 °C as illustrated for OBCet in Fig. 4. The amount of oxygen was approximately 7.5 mg/l (230 µM) in the untreated extracts and 8.5 mg/l (260 µM) in MVO. The autoclaving of 30 min consumed approximately 3 mg/l (90 µM) of the dissolved oxygen of the solutions, and the presence of ferrous ions further accelerated the oxygen consumption. This high magnitude of the consumption was demonstrated for enzymatic oxidation of galactoglucomannan resulting in a high degree of oxidized functional groups (>20%, Parikka et al., 2010). The values obtained here are realized to be only estimations due to the considerable viscosity changes during the treatments, the strong dependence of oxygen saturation on the temperature and on the available oxygen during the treatments (Naidja & Huang, 1999). However, the evident consumption supports the occurrence of oxidation reactions in heating.

3.3.5. *Oxidation reactions in relation to the thermal degradation of β-glucan*

The oxidation parameters – free radical and hydrogen peroxide formation, and oxygen consumption – increased consistently with molecular degradation in heating of the β-glucan solutions. Ferrous ion addition accelerated the formation of the radicals and the degradation of β-glucan. Furthermore, the viscosity loss was more intense in extract solutions with higher pro-oxidative activity determined as the formation of the reactive oxygen species. The correlation of the relative viscosity and molar mass decerase with the free radical formation was dipolar due to the more significant changes of the extracts containing ferrous ions when heated at 95 °C. When tested with a non-parametric correlation test, the order of radical formation activity in solutions explained over 80% of the viscosity and molar mass decrease ($r_{\mathrm{ho}} > 90\%$, $r_{\mathrm{K}} \geq 80\%$). This suggests that the free radical formation contributed to the molecular degradation of β-glucan during heating, and that oxidative cleavage is one of the key mechanisms in thermal degradation of β-glucan in aqueous solutions.

The higher pro-oxidant activity of the extract solution containing proteins, phytic acid and other impurities for the thermal degradation may be explained by the ability of such compounds to increase the pro-oxidativity of ferrous ions (Welch et al., 2002). In neutral water solutions, the redox potential of Fe^{3+}/Fe^{2+} (110 mV) is not actually sufficient to reduce the dissolved oxygen with redox potential of 150 mV as discussed by Welch et al. (2002). However, they suggested that iron may complex with chelates or other compounds such as proteins, which both occurred in relatively high concentrations in the extract solutions (Table 1), and act as a strong oxidant via Fenton chemistry (reaction (1)). In addition, OBC extract contained fatty acids such as oleic and linoleic acids (18:1 and 18:2) (Table 1), which are known to easily oxidize in heating, producing the oxidative hydroxyl and alkoxyl radicals. The added ferrous ions may also react by several ways affecting the intense of oxidation. The complexity of iron chemistry have created controversy of the particular oxidation activity of Fenton chemistry, and it is presumable that not only one universal oxidative Fenton mechanism occurs. For instance, Iron can complex with oxygen, and these ionic complexes can proceed to oxidative radicals, or oxidize the organic matter by non-radical mechanisms (Barbusinski, 2009).

3.4. *Inhibition of thermal degradation by antioxidants*

The protective activity of widely used food antioxidants in the thermal degradation of β-glucan in the solutions was studied. Ascorbic acid had no protective effect on β-glucan in the heating experiments. Ascorbic acid (0.2%, w/v), or ascorbate as it occurs at pH range 4.2–11.6, decreased the viscosity already without heating in all the solutions, and the decrease was promoted by heating (Table 3). Consistently to the viscosity, the molar mass decreased as well. In the MVO solution, the molar mass of β-glucan resulted in 70% of its original after the heat treatments (95 °C 30 min and 120 °C 5 min) in the presence of 1 mM of ascorbic acid despite the presence of ferrous ions. Ascorbic acid also induced molar mass decrease in storage temperature (+6 °C, 90% left of the original molar mass). In the extracts, the remaining viscosities were <10% of the original viscosities in the presence of ascorbic acid, independently of the reaction temperature (5 °C, 95 °C or 120 °C) and the molar masses decreased to be 13–35% of the original molar masses shown in Table 2. The Mark–Houwink's exponent α remained in the range of random coil (0.60–0.77) in the pure solutions (MVO) and in OBCet extract, thus the more intensive decrease in M_w in the presence of ascorbic acid indicated accelerated cleavage of β-glucan backbone. In OBC samples, the heat-treatment in the presence of ascorbic acid resulted in α-values of 0.2–0.3 indicating a highly compacted conformation. This may affect the viscosity decrease, like does the decrease in molar mass. Ascorbic acid is a widely used antioxidant, based on the relative stable ascorbyl radicals, which scavenge the oxidative free radicals. However, ascorbic acid is also an excellent reducing agent, and may, thus, behave as a pro-oxidant and oxidize polysaccharides in water solutions in the presence of transition metals (Fry, 1998). It can reduce and stabilise transition metals such as iron into the reduced form and reduce dissolved oxygen to hydrogen peroxide, which can further produce oxygen radicals by reaction (3). In addition, ascorbic acid may stabilise hydrogen peroxide, which easily decomposes under atmospheric conditions (Arts, Mombarg, van Bekkum & Sheldon, 1997). Oat β-glucan was shown to degrade by the addition of ascorbic acid consistently with the results of the present study in room and storage temperatures (+6 °C; Kivelä, Gates et al., 2009; Kivelä, Nyström et al., 2009). The pro-oxidant effect of ascorbate was not straightforwardly seen by the radical trapping experiments. The ascorbic acid addition solely without ferrous ions slowed down the radical formation in MVO, but in the extracts and with ferrous ions, the radical formation was significantly accelerated (Fig. 5). Andersen, Outrup and Skibsted (2000) demonstrated the pro-oxidativity of ascorbic acid as a boosted free radical formation in beer.

The effect of sulfite was partially inconsequence. At 95 °C, sulfite (0.2%, w/v) promoted the viscosity loss in the pure solution, but in the extracts no significant effect was obtained (Table 3). At 120 °C, sulfite clearly slowed down the viscosity loss of the extracts, but did not affect significantly in the pure solutions (Table 3). The rate of

Table 3
The remaining viscosity (%) of β-glucan solutions in the presence of ferrous ions (Fe^{2+}), ascorbic acid (AA) and/or sulfite (SO_3) after the heat-treatments of 95 °C for 30 min and 120 °C for 5 min. Different letters within a row denote a statistically significant difference at least 95% confidence level.

	Plain	$+Fe^{2+}$	+AA	$+Fe^{2+}$ + AA	$+Fe^{2+} + SO_3$	$+Fe^{2+}$ + AA + SO_3
95 °C 30 min						
MVO	88 ± 8^a	81 ± 5^b	45 ± 5^c	46 ± 1^c	54 ± 5^f	75 ± 6^{be}
OBC	88 ± 7^a	18 ± 14^b	3 ± 1^c	3 ± 1^c	23 ± 5^b	35 ± 6^d
OBCet	93 ± 6^a	23 ± 2^b	6 ± 1^c	6 ± 1^c	20 ± 3^b	42 ± 6^d
120 °C 5 min						
MVO	87 ± 4^a	67 ± 4^b	39 ± 2^C	39 ± 1^c	60 ± 3^b	87 ± 5^a
OBC	8 ± 1^a	3 ± 1^b	3 ± 1^b	3 ± 1^b	21 ± 1^c	37 ± 4^d
OBCet	21 ± 6^a	3 ± 1^b	3 ± 1^b	3 ± 1^b	18 ± 2^a	35 ± 3^d

radical formation slowed down in the extract solutions, which corresponds to the protective effect on viscosity. However, in the MVO solutions, where viscosity loss was accelerated, the radical formation was totally inhibited by the sulfite addition and no correlation between the solution instability and radical formation was found. The decreased/inhibited radical formation in the extracts is consistent with the previous results, which showed that the addition of sulfite in lager beer induced a lag phase into the amount of formed adducts and that sulfite was the most effective naturally occurring antioxidant in beer among several phenolic acids and ascorbic acid against free radical formation in beer (Andersen et al., 2000; Uchida & Ono, 1996). Autoclaving induced viscosity loss of guar gum solution was showed to be inhibited by sulfite (1%), but in the case of starch, sulfite acted concentration-dependently either as an antioxidant (appr. 0.3–1%, w/w, in 1% polymer solution) or as a pro-oxidant (appr. 0.005–0.3%). The two-directional effect was suggested to deal with the sulfite–oxygen ratio and the formation of sulfite radicals (Paterson, Hill, Mitchell & Blanshord, 1997). Sulfite may radicalize by the hydroxyl-radicals formed in the heating experiments producing oxidative $SO_3{}^-$-radicals. More importantly, this radical easily reacts with molecular oxygen, producing highly oxidative peroxyl radicals ($^\bullet SO_5{}^-$) (Neta & Huie, 1985). Thus, in one hand sulfite scavenges oxygen away from oxidation reactions, and in the other hand forms aggressive radicals with oxygen, which may create discrepancies to the results of antioxidativity of sulfite. Due to the high reactivity of sulfite with several compounds, the pro-oxidant nature of sulfites is evidenced only in model systems and not in real products such as beer or wine (Wedzicha, 2001, chapter 8). This is consistent with the current study, where the pure β-glucan solution suffered from the addition of sulfite, but in the presence of the co-extracted compounds, the addition of sulfite protected the solution from the heat-induced viscosity loss.

A clear effect against the thermal degradation was achieved using sulfite and ascorbic acid together. In all the solutions, ascorbic acid (0.2%, w/v) and sulfite (0.2%, w/v) used in combination eliminated the effect of Fe^{2+} and slowed significantly down the viscosity decrease. For example in the 120 °C-treated OBC and OBCet solutions, approximately 35% of viscosity remained with both sulfite and ascorbic acid addition as it was only 3% without the antioxidants (Table 3). The formation of free radicals was also totally inhibited in all the solution by adding ascorbic acid and sulfite together (Fig. 5). This is consistent with the inhibition ability of a mix of sulfite and a phenolic compound against autoclaving induced viscosity loss of galactomannan (Hill & Gray, 1999; Paterson et al., 1997). The sulfite radicals, especially after the reaction with oxygen, rapidly ($k = 1.4 \times 10^8\ M^{-1}\ s^{-1}$) react with ascorbate producing ascorbyl radicals and decays bythemselves (Neta & Huie, 1985). The antioxidant nature of ascorbate will dominate, as it has been quickly converted to the ascorbyl radicals, which can scavenge the hydroxyl and other oxidizing radicals formed in the heating experiments. The results here show the complexity of managing oxidation reactions in systems, where impurities, air and heating energy are present. However, the synergistic effect of sulfite and ascorbic acid to protect β-glucan from autoclaving induced degradation encourages to further work in this area.

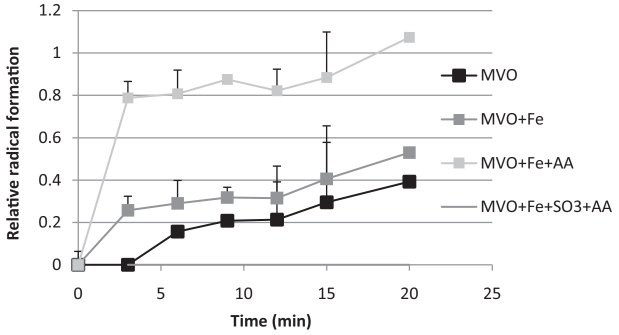

Fig. 5. Effect of ascorbic acid (AA) on the relative radical formation (sample signal intensity in relation to stable radical TEMPO) in MVO solution with ferrous ions at 95 °C. The addition of AA with sulfite (SO_3) totally inhibited the free radical formation, thus being poorly visible in the figure.

4. Conclusions

Soluble oat β-glucan degraded in thermal treatments and the degradation was significant at the 120 °C. Viscosity decreased irreversibly, parallel to molar mass, and the degradation increased with increasing exposure time. Oxidation evidently played a role in the thermal degradation, since the addition of Fe^{2+}-ions accelerated the thermal degradation, strongly oxidative free radicals were formed and oxygen consumed in parallel to the scission of β-glucan backbone during the heat-treatments. The management of the oxidation reactions during the heating in these semi-dilute solutions proved to be challenging. Ascorbic acid clearly acted as a pro-oxidant and sulfite slowed down the viscosity loss only in the extracts. However, the use of ascorbic acid in combination with sulfite showed promising protective effect towards the thermal degradation of β-glucan. According to the results here, the intensity of thermal degradation in solution is affected by co-extracted compounds such as phtates, proteins and minerals, and the controlling of non-enzymatic reactions is crucial in management of the molecular stability of cereal β-glucan in products and processing.

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