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Oxidation of oat β-glucan in aqueous solutions during processing

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ABSTRACT

The study investigated carbonyl group formation along the chain and the chain cleavage of cereal β-glucan during heat treatments, high pressure homogenisation, cold storage and ascorbic acid treatment of aqueous solutions of this soluble dietary fibre. The carbonyl group content and its distribution along the chain were simultaneously determined with the chain cleavage using a HPSEC/labelling method, originally developed for water-insoluble cellulose. Ascorbic acid treatment resulted in a relatively high degree of carbonyl content and extensive degradation of β-glucan, even in concentrations typically found in foods. The thermal oxidation of the β-glucan was considerable at 120 °C in a β-glucan solution with co-extracted compounds from oat ingredient, and in the highly purified solutions in presence of ferrous ions. Oxidation also probably contributed to the molecular properties during high pressure homogenisation, even thou the main degradation mechanism is the hydrolysis caused by mechanical energy. In addition to the cleavage of the β-glucan chain, the formation of compact, high molar mass species or molecule clusters were obtained in the study after ascorbic acid, heat (120 °C) and homogenisation treatments.

1. Introduction

General interest in (1 → 3),(1 → 4)-β-D-glucan (hereafter β-glucan), the main soluble dietary fibre component in oats and barley, has increased in recent decades due to its proven health benefits in humans (FDA, 1997; EFSA, 2009, 2010). The health promoting effects are often related to the ability of this linear glucan to form and increase the viscosity of digesta (Wood, 2010), thus slowing down or inhibiting the absorption of low molar mass compounds related to diseases such as diabetes, coronary heart disease and obesity. The β-glucan molecule consists of glucose units, which are linked by 1 → 3-β-linkages and 1 → 4-β-linkages. The flexible 1 → 3-β-linkages interrupt rigid cellulose-like regions formed by conjunctive 1 → 4-β-linkages, which increases the solubility of this high molar mass glucan chain in water, and further contributes to the viscosity forming ability of cereal β-glucans (Buliga, Brant & Fincher, 1986). The viscosity of the solution strongly depends on the molar mass, solubility and extractability of β-glucan. All these properties are easily altered in the aqueous processing of oats (Åman, Rimsten & Andersson, 2004; Tosh et al., 2010).

Recently, the functionality of oat β-glucan was demonstrated to be influenced by oxidation (Kivelä, Gates & Sontag-Strohm, 2009, Kivelä, Nyström, Salovaara & Sontag-Strohm, 2009; Kivelä, Sontag-Strohm, Loponen, Tuomainen & Nyström, 2011; Park, Bae, Lee, & Lee, 2009). Oxidation is a widely studied degradation mechanism of other polysaccharides such as cellulose, but was previously neglected in investigations of cereal β-glucan stability. The viscosity of β-glucan solutions and molar mass of β-glucan were found to significantly decrease following the addition of Fe^{2+} ions and/or ascorbic acid, which was associated with Fenton chemistry and β-glucan oxidation (Kivelä, Nyström, et al., 2009). Oxidative reactions were also related to the thermal degradation of β-glucan in the aqueous environment, since strongly oxidative free radicals were formed during heating (Kivelä et al., 2011; Robert, Barbati, Ricq & Ambrosio, 2002).

In general, the oxidation of organic matter is based on the production of highly reactive hydroxyl radicals. One of the widely accepted sources of hydroxyl radicals in biological systems is connected with Fenton chemistry. The system is generally known as the Haber–Weiss cycle, which comprises the reduction of iron or copper (Reaction (1)) and the Fenton reaction (Reaction (2)) (Haber & Weiss, 1932; Koppenol, 2001). In the Fenton reaction, hydrogen peroxide is reduced in a transition metal-catalyzed reaction, producing hydroxyl radicals. Reducing agents such as ascorbate (ascorbic acid) can reduce the catalysts iron and copper (Reaction (4)) and reduce molecular oxygen to hydrogen peroxide (Reaction (5); Buettner & Jurkiewicz, 1996). In addition, ascorbic acid can stabilise hydrogen peroxide (Arts, Mombarg, vanBekkum & Sheldon, 1997), thus enabling and promoting hydroxyl radical production. The oxidative role of Haber–Weiss/Fenton processes in biological conditions is generally known, but the mechanisms and reaction pathways are still under discussion (Barbusinski, 2009; Koppenol, 2001; Liochev & Fridovich, 2002). The production of highly oxida-

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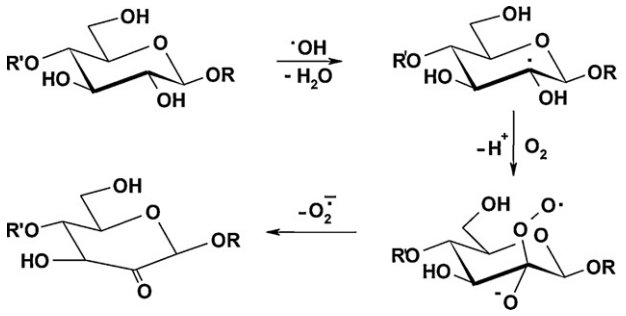

Scheme 1. Formation of carbonyl structures as a consequence of hydrogen abstraction by hydroxyl radicals (modified from Gratzl, 1987).

tive ferryl ions, especially in the presence of low amounts of hydrogen peroxide, has been highlighted (Barbusinski, 2009).

$$Cu^{+}/Fe^{2+} + O_2 \rightleftarrows Cu^{2+}/Fe^{3+} + O_2^{\bullet -} \quad (1)$$

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

$${}^{\bullet}O_2^{-} + H_2O_2 \rightarrow {}^{\bullet}OH + OH^{-} + O_2 \quad \text{(netreaction)} \quad (3)$$

$$AH_2 + 2Cu^{2+}/Fe^{3+} \rightarrow A + 2H^{+} + 2Cu^{+}/Fe^{2+} \quad (4)$$

$$AH_2 + O_2 \rightarrow A + H_2O_2 \quad (5)$$

Hydroxyl radicals non-selectively attack macromolecules at diffusion- and pH-dependent rates (Gilbert, King & Thomas, 1984; von Sonntag, 1980). The radicals attack the molecules in their immediate vicinity, since their half-life time is about 1 ns. Due to this short lifetime, the localisation of metals is one of the dominant factors in the Fenton chemistry-derived oxidation of macromolecules. Hydroxyl radical attack initiates chain reactions by abstracting the hydrogen from C–H moieties, which leads to the formation of alkoxyl radicals. These alkoxyl radicals, which are radicalised chains in the case of polysaccharides, can react with atmospheric oxygen, leading to a peroxyl radical intermediate and eventually forming a carbonyl group, as illustrated in Scheme 1. In the case of polysaccharides, depending on which carbon is attacked, this can lead to the conversion of the hydroxyl groups of the anhydroglucose to carbonyl functionalities, and in case of C6 also to carboxyl groups, as well as cleavage of the glycosidic bond and/or the formation of numerous degradation products (Arts et al., 1997; Gratzl, 1987; Potthast, Rosenau & Kosma, 2006). Hydrogen abstraction is non-specific in an anhydroglucose ring (von Sonntag, 1980), but attacks on the carbons involved in glycosidic bonds may be hindered, as shown by dextran (Gilbert et al., 1984).

The aim of this study was to investigate the oxidation of the functional groups of oat β-glucan during the processing of β-glucan solutions. The treatments were selected based on our recent studies on the non-enzymatic degradation of β-glucan relating to the processing of liquid fibre-rich products, and included heat treatments at 95 °C and 120 °C, high pressure homogenisation and ascorbic acid treatment. The effect of the co-extracted compounds of oat β-glucan and the addition of ferrous ions on the intensity of oxidation were also investigated. Oxidation was studied by modifying a labelling technique for carbonyl groups previously developed for cellulose (Röhrling et al., 2002a,b) to fit the β-glucan substrate. This sensitive method allows the simultaneous determination of the molar mass distribution and the quantification and distribution of carbonyls that include reducing end groups, as well as additional keto and aldehyde groups, along the polymer chains.

2. Materials and methods

2.1. β-Glucan solutions

Native β-glucan was extracted from an oat bran concentrate (Oat Well 14%, Swedish Oat Fibre, Sweden) with a gentle method. The bran was dispersed in water at a ratio 10 g bran per 100 mL water (except in homogenisation experiments, in which the ratio was 6 g per 100 mL), shaken at 40 °C for 30 min, and the supernatant was collected after centrifugation for 10 min at 16,000 g. The supernatant was used as a solution of native β-glucan (NBG) prior to the treatments. After the treatments, 99% ethanol (Altia, Finland) was added to the samples in the ratio 1:1 and vigorously shaken. The formed precipitate was collected (NBGp), washed thoroughly twice with ethanol by shaking in tubes and dried overnight at 60 °C.

Commercial oat β-glucan (medium viscosity 99%, Megazyme International, Bray Business Park, Ireland) was dissolved in water by wetting it with 99% ethanol, dispersing it into deionised water and mixing it for 3 h at 80 °C with a magnetic stirrer. The solution of commercial oat β-glucan is referred to here as OBG.

2.2. Solutions of reduced β-glucan

The original OBG solution was treated with sodium borohydride ($NaBH_4$) in order to reduce the already existing carbonyl groups (incl. reducing ends) from the initial materials before treating them with potential oxidation agents and processes. An aqueous solution of $NaBH_4$ was added to the β-glucan solution so that the resulting concentration of $NaBH_4$ was 100 μmol/mL in the solution containing 10 mg/mL of β-glucan. Before adding $NaBH_4$, the pH of β-glucan solutions was adjusted to 10 using 0.1 M sodium hydroxide. After a reaction time of 24 h, β-glucan was precipitated from the aqueous solution with ethanol, washed thoroughly with 99% ethanol and redissolved in water.

2.3. Quantification of β-glucan, proteins and minerals in β-glucan solutions

Characterisation of the sample materials was performed from freeze dried native extract, purified precipitate (NBG) and commercial oat β-glucan (OBG). Proteins were quantified by the Dumas combustion method (N × 6.25) and the β-glucan content was determined in triplicate with an enzymatic method (Approved Method 32-23, AACC 2000) using a Megazyme kit BBG (Megazyme International, Bray Business Park, Ireland). The mineral content was analysed after wet digestion (HNO_3) using an inductively coupled plasma-mass spectrometer (ICP-MS, PerkinElmer Elan 6000) with an external standard method (in-laboratory flour reference and standard reference material 1567 of NIST).

2.4. Solution treatments

β-Glucan solutions were subjected to the following treatments to cause the non-enzymatic degradation of β-glucan: heat treatment, high pressure homogenisation, ascorbic acid treatment, ferrous ion addition and cold storage. An oil bath was used to heat the solution to 95 °C, 120 °C was reached in an autoclave, and a sensor in the solutions was used to monitor the temperature. Transition metal ions were added as $FeSO_4{\cdot}7H_2O$ in the heating experiments on OBG at concentrations of 0.1 mM and 1 mM. High pressure homogenisation was performed with a microfluidizer (M-110Y Microfluidizer® Processor, Newton, USA) at the lowest and highest pressures possible (300 and 1000 bar) in the continuous mode at a constant exposure time (5 min). The effects of ascorbic acid were examined at concentrations of 2 mM, 10 mM and 50 mM, since the typical concentration of ascorbic acid in juices is

30–40 mg/100 mL (1.7–2.2 mM) and the recommended consumption of ascorbic acid is 60 mg per day. Ascorbic acid was added as a solid to the β-glucan solutions, dissolved and allowed to react over 4 h (NBG) or 4 days (OBG) at room temperature. All the ascorbic acid treated samples were precipitated and washed thoroughly, since ascorbic acid interferes with the CCOA protocol by consuming the reagent.

2.5. Fluorescence labelling

CCOA (carbazole-9-carboxylic acid [2-(2-aminooxyethoxy)ethoxy]amide) was added to the treated OBG solutions and the treated + purified NBG solutions to label the carbonyl groups for fluorescence detection, as previously reported for cellulose (Potthast et al., 2003; Röhrling et al., 2002a,b). The method was modified so that the labelling was performed under homogeneous conditions in a buffer. The dried samples were dissolved in water (10 mg/mL OBG, 1 mg/mL NBG) by shaking them overnight. A CCOA stock solution was prepared by dissolving 3.3 mg/mL CCOA label in a zinc acetate buffer at pH 4. The CCOA stock solution and the sample solutions were combined in the ratio 0.3 mL:0.5 mL, resulting in a CCOA concentration of 1.25 mg/mL in the samples. The solutions were agitated in a water bath for 7 days at 40 °C. Thereafter, unreacted label was removed by precipitation of the β-glucan with ethanol (60%) and thorough washing with 99% ethanol. Immediately after washing the sample, the β-glucan was dissolved in 1 mL DMAc/LiCl (0.9%, w/v) by shaking at room temperature overnight. NBG samples were diluted with 0.250 mL DMAc/LiCl (9%, w/v), which resulted in a LiCl concentration of 2.5% and a clear solution. All the samples were filtered through 0.45 μm syringe filters prior to HPSEC analysis.

2.6. HPSEC

HPSEC analysis of the samples was carried out using DMAc/LiCl (0.9%, w/v) as the eluent, filtered through a 0.02 μm filter. The equipment consisted of an autosampler module (Hewlett Packard 1100 series), a Kontron pump, a Dawn DSP multiple-angle laser light scattering (MALLS) detector with an argon ion laser (λ_0 = 488 nm) (Wyatt Technology Corporation), a refractive index detector (Shodex RI-71) and a fluorescence detector (Shimadzu, RS-535). The light scattering detector included 18 scattering angles. Four serial SEC columns (Varian, PLgel 20 μm Mixed-ALS 300 mm × 7.5 mm), an injection volume of 100 μL and a flow rate of 1.00 mL/min were used as the chromatographic parameters. The molecular weight distribution, root mean square radius and other polymer-related parameters were calculated by using a dn/dc value of 0.136, which was determined for cellulose in DMAc/LiCl (0.9%, w/v) at 25 °C and 488 nm at BOKU (Vienna, Austria). The CCOA bound to carbonyl groups was detected at 290 nm excitation, 340 nm emission. The total carbonyl content (μmol/g) was calculated towards six cellulose standards with known carbonyl group contents labelled in the same series (Röhrling et al., 2002a,b), and is presented as $c(\text{carbonyl})_{\text{total}}$. The content of carbonyl groups formed along the chain was determined by estimating the number of reducing end groups as a reciprocal of the number-average molar mass (M_n) as follows:

$$c(\text{carbonyl}) = c(\text{carbonyl})_{\text{total}} = \frac{1}{M_n} \times 10^6$$

In the case of reduced ($NaBH_4$)-treated samples, the $c(\text{carbonyl})_{\text{total}}$ was used, since in theory no reducing ends occurred in the starting material. In practice, the untreated OBG also gave a fluorescent signal, as reported below in the results and discussion sections. Due to the estimations, the results for carbonyl group contents along the β-glucan chain are only indicative. However, due to the sensitive and selective analysis method for the keto and aldehyde groups, interpretation of the occurrence of β-glucan oxidation during the treatments is valid.

2.7. Viscosity

Rheological measurements were carried out for the aqueous solutions with a ThermoHaake RheoStress 600 rheometer (Thermo Electron GmbH, Dreieich, Germany) using a cone and plate geometry (titanium, 35 mm, 2°). Viscosity properties were measured over a shear rate range of 0.3–300–0.3 s^{-1} and characterised in duplicate with the Ostwald de Waele power law at +20 °C. The results were presented as an average of three individual series of apparent viscosities (10 s^{-1}).

3. Results and discussion

3.1. Material characterisation and dissolution

The studies were carried out with highly purified oat β-glucan (OBG) free of proteins and other constituents that interfere the labelling, or with native β-glucan (NBG) in an aqueous extract in the presence of the co-extracted impurities. The latter approach modelled the reactions in food systems (Table 1). The extract contained approximately half of its dry weight of β-glucan, and the rest consisted mostly of proteins, minerals including phytate (Table 1), and other low molar mass compounds. Because proteins at high concentrations may interfere with determination of the carbonyl content by consuming the excess of CCOA, the NBG samples were purified by precipitating β-glucan from the extract before labelling (NBGp). The protein content decreased from 15% to below 5% (w/w) as a result of the purification step (Table 1).

The potential oxidative treatments were performed in aqueous solutions. However, since water is not always the best solvent in analysis due to the aggregation properties of β-glucan (Li, Wang, Cui, Huang, & Kakuda, 2006; Li, Cui, Wang & Yada, 2011), high performance size exclusion chromatography (HPSEC) was performed in an organic solvent DMAc/LiCl. Dry β-glucan powder was insoluble in DMAc/LiCl (0–6%, w/v), but when OBG powder was wetted with ethanol before dissolution in DMAc/LiCl (0.9%, w/v), a clear solution was obtained after shaking overnight. NBG formed clear solutions after the LiCl concentration was increased to 2.5% (w/v). The weight-average molar masses (M_w) of β-glucan in untreated OBG and NBG were 260,000 g/mol and 1600,000 g/mol, respectively. These are similar to previously reported values analysed in water solutions by HPSEC (Kivelä et al., 2011; Lazaridou & Biliaderis, 2007; supplier Megazyme International Ireland Ltd). In previous studies, aggregation has been reduced by different aqueous solvents such as alkaline (NaOH, SDS; Li et al., 2006; Wu et al., 2006) and acid solutions, and the traditional cellulose solvents cadoxen (Li et al., 2011) and cuoxam (Grimm, Krüger, & Burchard, 1995). Organic solvents (DMSO) have been used to dis-

Table 1
Characterisation of the studied β-glucan solutions NBG and OBG. OBG is for commercial oat β-glucan, NBG for a β-glucan extract from oat bran concentrate and NBGp for an ethanol precipitated β-glucan from the extract. The protein, mineral and beta-glucan content is determined as the proportion of the dry matter (d.m.) of the solutions.

	OBG	NBG	NBGp
Beta-glucan (%, w/w of d.m.)	99	47 ± 4	87 ± 3
Fe (mg/kg of d.m.)	130 ± 2	25 ± 2	40 ± 5
Cu (mg/kg of d.m.)	3 ± 1	23 ± 1.	3 ± 1
Zn (mg/kg of d.m.)	30 ± 2	45 ± 3	22 ± 4
P (mg/kg of d.m.)	3900 ± 100	19000 ± 1000	9000 ± 1000
Proteins (%, w/w of d.m.)	1 ± 1	15 ± 3	4 ± 1

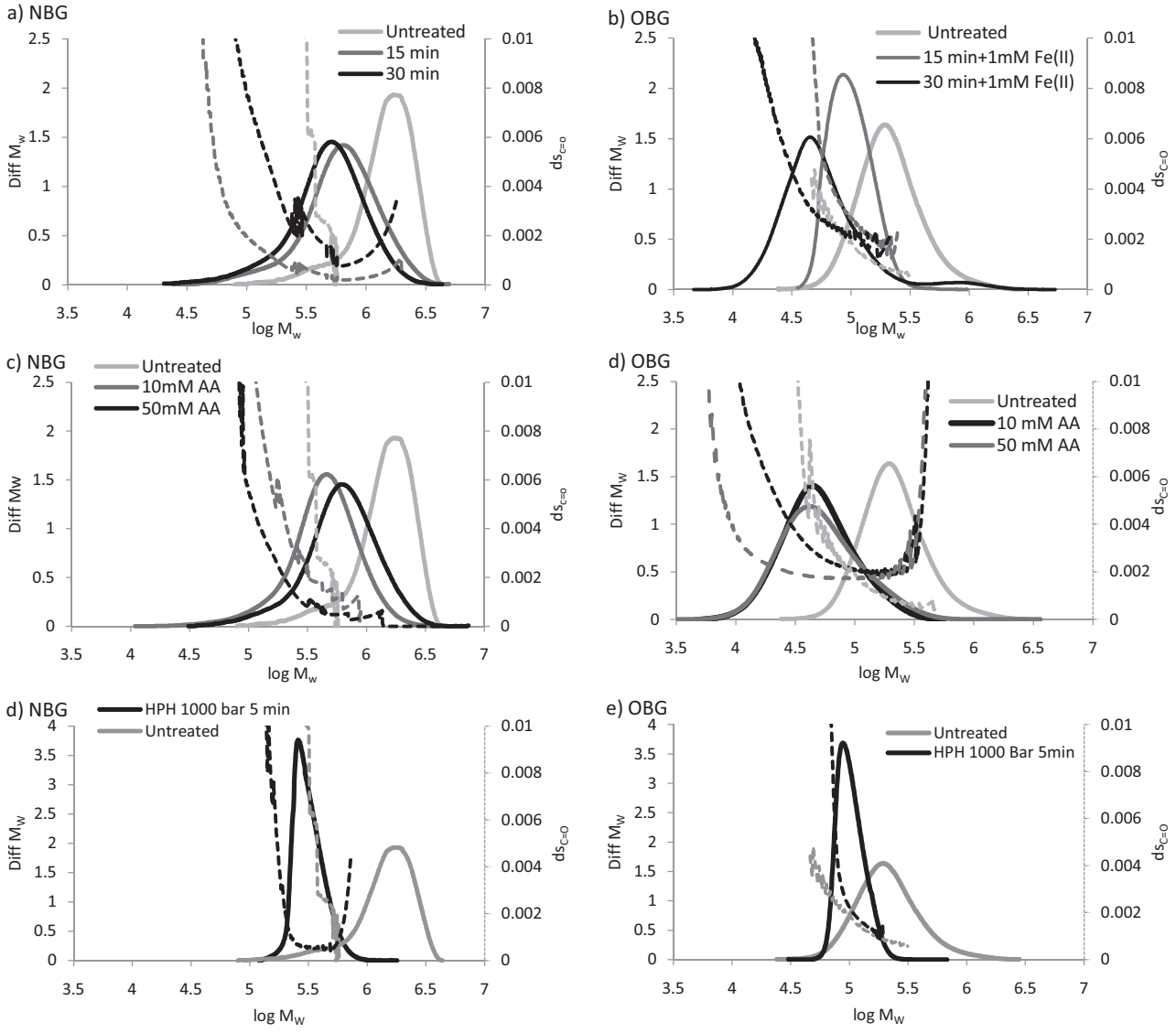

Fig. 1. Molar mass (M_w, right axis and solid line) and carbonyl group substitution ($ds_{C=O}$, left axis and dashed line) of β-glucan in (a) heat-treated NBG (120 °C for 15 min and 30 min), (b) heat-treated OBG after (120 °C for 15 min and 30 min in presence of 1 mM $FeSO_4$), (c) ascorbic acid (AA) treated NBG (reaction time 4 h), (d) ascorbic acid treated OBG (reaction time four days), (e) high pressure homogenised (HPH) NBG and (f) high pressure homogenised OBG.

solve cereal β-glucan prior to NMR analysis (Ghotra, Vasanthan & Temelli, 2008). DMAc/LiCl is known to reveal cellulosic aggregates, and thus, according to the similarity of the molar masses obtained with DMAc/LiCl and previously in aqueous solvents, the results support the previous demonstration of a negligible degree of aggregation of β-glucan under HPSEC conditions (Li et al., 2006).

3.2. Oxidation of β-glucan during heating

Heating for short times (s/min) at temperatures of 100 ± 30 °C is typically used in the pasteurisation and sterilisation of food. The molar mass of β-glucan decreased in both test solutions, OBG and NBG, when heated at 120 °C (Table 2). This is consistent with the previously reported results of backbone cleavage of oat β-glucan at 120 °C (Kivelä et al., 2011; Wang, Wood & Ross-Murphy, 2001). The lower heating temperature (95 °C) also slightly reduced the molar mass of β-glucan in both solutions. However, the effect of thermal degradation at 95 °C observed here was negligible when compared to the molar mass decrease previously shown for other water-soluble polysaccharides such as κ-carragenan (Lai, Lii, Hung & Lu, 2000) or galactomannan (Kök, Hill & Mitchell, 1999), and indicates β-glucan to be relatively resistant to thermal treatment.

The molar mass of β-glucan in the extract (NBG) steeply decreased during the treatment at 120 °C for 30 min (Table 2 and Fig. 1a), while in the highly purified solution (OBG), the same treatment caused only a slight decrease in the molar mass of β-glucan (Table 2 and Fig. 1b). This principally suggests that the differences in the original molar mass of the solutions NBG and OBG, the concentration or the co-extracted compounds had a role in the rate of thermal degradation. The purification of the NBG samples by ethanol precipitation (NBGp, Table 1) demonstrated that the role of the co-extracted compounds was significant, since the NBG and NBGp differed only in the presence of proteins, minerals and other co-extracted compounds, and not in molar mass or concentration. The autoclaving treatment (120 °C, 15 min) reduced the molar mass of NBG from 1,600,000 g/mol to 700,000 g/mol, whereas the treatment of NBGp with the same original molar mass resulted in 1,400,000/mol (Table 2, NBGp in parenthesis). The co-extracted compounds may contribute to the oxidative activity of the solutions, which was previously shown by the higher degree of

Table 2
Indicative concentrations of carbonyls (marked as c=o) along the β-glucan chain, and molar masses (M_w) of β-glucan after the ascorbic acid, heat- and high pressure homogenisation (HPH) treatments of OBG (a solution of purified β-glucan) and NBG (an extract of native β-glucan). The asterisk (*) means that the values in parenthesis are for the OBG, treated with $NaBH_4$ before the heating and ascorbic acid treatments. The double asterisk (**) means that the values in parenthesis are for NBG, which was purified (i.e., the NBGp sample) before the heat-treatments.

Added $FeSO_4$ (mM)	Untreated	Heating						Ascorbic acid addition[a]				HPH
		95 °C/30min		120 °C/30min		120 °C/15min		2 mM	l0 mM		50 mM	100 MPa
	–	–	1	–	0.1	1	1		0.1	–	–	–
OBG												
$c_{c=O}$ (μmol/g)	1 (1)*	1	7	2	3	5(6)*		16	7	22 (20)*	17	10
M_w (kg/mol)	260 (275)*	250	270	240	220	100(140)*		100	60	60 (70)*	70	100
NBG												
$c_{c=O}$ (μmol/g)	1	2		12			7 (4)**	4		9	6	5
M_w (kg/mol)	1600	1430		600			730 (1400)**	740		550	750	190

[a] Reaction time in OBG 4 days and in NBG 4 h.

hydroxyl radical formation in the extract solution than in the highly purified solution (Kivelä et al., 2011). Indications of the higher oxidative activity of NBG were also observed in the present study, since the carbonyl group formation was higher in the NBG samples than in the purified OBG and NBGp samples during the treatments at 120 °C (Table 2). The co-extracted compounds to be considered are especially transition metals, phosphorous compounds and proteins, which have all been reported to associate with cereal β-glucan (Ghotra, Vasanthan, Wettasinghe & Temelli, 2007; Mälkki, 1992; Platt and Clydesdale, 1984), and were present in relatively high amounts in NBG (Table 2). In addition, the oxidation catalysts, i.e., transition metals, most likely associate via chelates and proteins with β-glucan in cereals (Persson, Nyman, Liljeberg, Önning & Frolich, 1991). Furthermore, as the formation of complexes with chelates and proteins enhances the reduction power of at least iron (Welch, Davis & Aust, 2002), these co-extracted compounds may contribute to the recorded oxidative activity of NBG.

No clear evidence of the oxidation of highly purified β-glucan in OBG during the heat treatments at 120 °C was obtained (Table 2), even though hydroxyl radical formation was previously induced by thermal treatment in pure solutions (Kivelä et al., 2011). When the role of oxidation of this pure β-glucan was further studied in the presence of an added oxidation catalyst, i.e., ferrous ions, clear degradation of β-glucan was observed. The 30 min autoclaving treatment with $FeSO_4$ at concentrations of 1 mM and 0.1 mM reduced the molar mass of β-glucan from 260,000 g/mol to approximately 100,000 and 220,000 g/mol, respectively, and without the addition of ferrous ions to 240,000 g/mol (Table 2). The carbonyl content slightly increased as increasing amounts of ferrous ions were added (Table 2), indicating the oxidation of the functional groups of β-glucan and the contribution of oxidative cleavage during the heat treatments. When the exposure time was shorter (15 min) at 120 °C in the presence of 1 mM $FeSO_4$, the degree of carbonyl formation was significantly higher, and comparable with the carbonyl concentrations recorded from e-beamed cellulose (Potthast et al., 2003). The concentration of carbonyls was considerably higher after this shorter exposure time than after the longer (30 min) treatment time with a more intense molar mass loss of β-glucan (Table 2 and Fig. 1b). This suggests that ferrous ions induced the oxidative cleavage of β-glucan, which assumingly progressed to further oxidation products during the longer exposure times. Under the milder conditions (30 min at 95 °C) in the presence of 1 mM $FeSO_4$, a significantly greater quantity of carbonyl groups was formed, but the molar mass did not significantly decrease (Table 2). This indicates the formation of stable carbonyl groups along the β-glucan chain, which do not lead to fragmentation or hydrolysis of the chain (von Sonntag, 1980), but alter the properties of β-glucan.

The heat treatment at 120 °C in the presence of 1 mM $FeSO_4$ was repeated with the $NaBH_4$-treated OBG solution, since $NaBH_4$ treatment reduces the existing carbonyls, i.e., reducing end groups, and possible oxidized functional groups of the untreated β-glucan. The reduction step enabled the investigation of free radical-induced oxidative cleavage instead of depolymerisation initiated by reducing end groups, fragmentation by β-elimination, or acid hydrolysis, the intensity of which is higher in the presence of carbonyls in the chain. The molar mass of the $NaBH_4$-treated β-glucan decreased and the degree of carbonyl groups increased similarly to the non-reduced β-glucan during autoclaving (Table 2, $NaBH_4$-treated in parentheses). This indicates that the cleavage was not a result of heat-induced hydrolysis, but the treatments at 120 °C oxidized β-glucan, causing cleavage and carbonyl group formation along the β-glucan chain.

3.3. Oxidation of β-glucan by the addition of ascorbic acid

Ascorbic acid, or ascorbate when it occurs in the pH range 4.2–11.6, is a widely used antioxidant based on relatively stable ascorbyl radicals, which scavenge oxidative free radicals. However, before ascorbyl radical production, ascorbic acid acts as an excellent reducing agent, and may thus behave as a pro-oxidant in aqueous solutions in the presence of transition metals, as shown in Reactions (4) and (5).

In the present study, ascorbic acid treatments significantly reduced the molar mass of β-glucan in both solutions (Table 2). In the NBG solution, the molar masses after a 4-h reaction time decreased from 1,600,000 g/mol to 800,000 g/mol, 550,000 g/mol and 750,000 g/mol as a result of 2 mM, 10 mM and 50 mM ascorbic acid addition, respectively (Table 2 and Fig. 1c). In OBG, the addition of 10 mM of ascorbic acid resulted in 60,000 g/mol and the addition of 50 mM in 70,000 g/mol after a 4-day reaction time (Table 2 and Fig. 1b). Simultaneously with the molecular degradation, the degree of carbonyl group formation significantly increased, in the order of 10 mM > 50 mM > 2 mM of ascorbic acid addition in the NBG extract sample, and 10 mM > 50 mM in the OBG sample (Table 2), indicating an oxidation process induced by ascorbic acid. The concentration of carbonyls was significantly higher in the ascorbic acid-treated highly purified β-glucan (≈20 μmol/g) after a reaction time of four days, and 5–10 μmol/g in the extract after 4 h of reaction. The formation of 15 oxidized groups/1000 sugars of xyloglucan has been reported when treated with ascorbate (20 mM), Cu_2SO_4 (50 mM) and hydrogen peroxide (10 mM) for 8 h (Fry, Dumville, & Miller, 2001). This gives an overall degree of substitution of approximately 0.005, and further ≈30 μmol/g, which is consistent with the results of the present study if the differences in study conditions are taken into account. For example, hydrogen peroxide enhances the loss of viscosity, and copper is a more efficient catalyst than iron (Buettner & Jurkiewicz, 1996; Fry, 1998). Interestingly, ascorbic acid not only increased the carbonyl group content of OBG, but caused a sharp increase in the degree of substitution in the highest molar mass species (Figs. 1c and d). The reduction treatment of OBG before

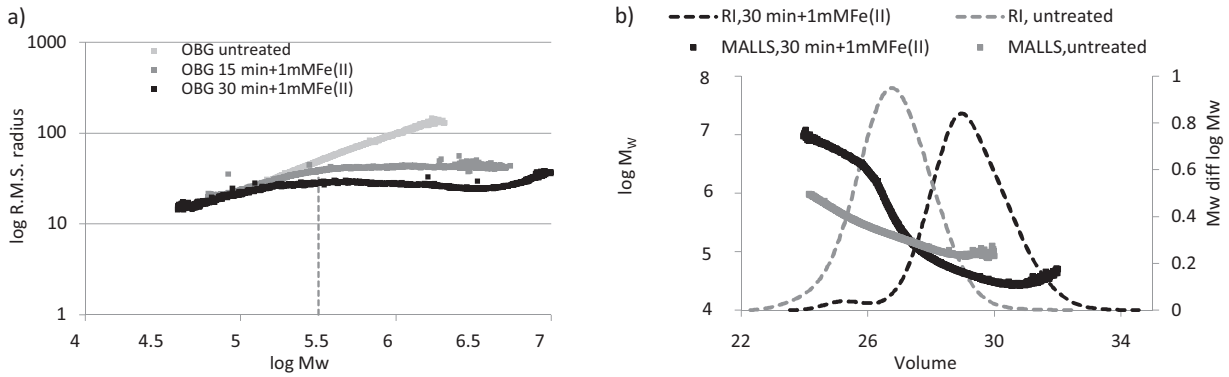

Fig. 2. (a) A conformation plot of β-glucan after 15 min (dark gray line) and 30 min (black line) heating at 120 °C with 1 mM $FeSO_4$ and (d) RI (dashed lines) and MALLS-signals (solid lines) after the 30 min heating at 120 °C in presence of 1 mM $FeSO_4$ (black line).

treatment with 10 mM ascorbic acid did not change the obtained molar mass decrease or carbonyl group formation (Table 2, $NaBH_4$-treated in parentheses). The carbonyl group contents as well as the weight-average molar mass were similar with and without the $NaBH_4$ treatment. This implies that ascorbic acid can induce free radical-mediated oxidation reactions, resulting in the oxidation of polysaccharides in aqueous solutions, as previously reported for oat β-glucan (Kivelä, Gates, et al., 2009; Kivelä, Nyström, et al., 2009) and for other water-soluble glucans such as cassava starch (Vallès-Pàmies et al., 1997), pullulan (Crescenzi, Belardinelli & Rinaldi, 1997), xyloglucan (Fry, 1998), chitosan (Zoldners, Kiseleva & Kaiminsh, 2005) and carboxymethyl cellulose (Crescenzi et al., 1997; Fry, 1998).

The slightly higher degree of molar mass decrease as well as the degree of oxidation by 10 mM compared to 50 mM of ascorbic acid addition, seen in both β-glucan solutions, may be due to the cross-over effect reported elsewhere (Rees & Slater, 1987). The cross-over effect derives from the dual nature of ascorbic acid and the simultaneous formation of antioxidative ascorbyl radicals, as well as pro-oxidative metal ions and hydrogen peroxide. At higher concentrations, the ascorbyl radicals are formed in sufficient amounts to scavenge the hydroxyl radicals produced by the reducing power of ascorbic acid, and thus terminate the oxidation chain reactions (antioxidant). At lower concentrations, the reducing power of ascorbic acid dominates, resulting in hydroxyl radical formation without a sufficient concentration of the scavengers (pro-oxidant).

3.3.1. *Oxidation of β-glucan during high pressure homogenisation*

Homogenisation of β-glucan significantly reduced its molar mass in both solutions (Table 2 and Fig. 1e and f). In OBG, the molar mass decreased to 100,000 g/mol and in NBG to 200,000 g/mol after 5 min of continuous exposure to 1000 bar, which agrees with previously reported results of the cleavage of β-glucan during high pressure homogenisation (Kivelä, Pitkänen, Laine, Aseyev & Sontag-Strohm, 2010). The degradation of polysaccharides in high pressure homogeniser treatment (microfluidizer) is mainly due to turbulent and elongational forces, to which the polysaccharide chains are respectively subjected under high hydrodynamic pressure and high velocity in the capillaries of the homogeniser (Stang, Schuman & Schulbert, 2001). These forces are dependent on processing parameters such as the chamber pressure, equipment parameters and solution parameters such as viscosity. Since there was only slight variation in the original viscosity of the solutions of OBG ($\eta_{app(10\ 1/s)} \approx 50$ mPa s) and NBG ($\eta_{app(10\ 1/s)} \approx 150$ mPa s), the similar sizes of the resulting products were expected.

Oxidation may play a role in high pressure homogenisation treatments using hydrodynamic cavitation, which may also to contribute to particle disruption in high pressure homogenisers (Stang et al., 2001). Cavitation, the shock waves produced by bubbles in a liquid, can induce homolytic fission of water molecules to hydroxyl and hydrogen radicals and thus cause oxidation (Halliwell & Gutteridge, 2007). Hydrodynamic cavitation in high pressure homogenisers has been demonstrated to be effective in oxidising low molar mass compounds (Gogate & Kabadi, 2009; Gogate & Pandit, 2005). In addition, free radicals have been demonstrated to form simultaneously with polysaccharide degradation when homogenising with ≈ 30 bar (Lander et al., 2000). The present study implied the formation of carbonyl groups along the β-glucan chain during high pressure homogenisation. The concentration of carbonyl groups was low (5–10 μmol/g, Table 2), but they occurred throughout the molar mass distribution in NBG (Fig. 1e). This indicated the formation of stable carbonyl groups along the chain and the contribution of oxidative reactions in homogenisation. As a conclusion, oxidation was not the predominant degradation force compared to the mechanical energy input and capillary effect of high pressure homogenisation, but oxidation reactions may, however, contribute to the properties of the homogenised β-glucan molecule.

3.4. *Changes in β-glucan conformation during treatment*

The influence of the treatments on the conformation of β-glucan was evaluated by a slope $\log M_w$ as a function of $\log R_{r.m.s.}$ In the NBG solution, the slope of the conformation plot was ≈0.7 for β-glucan before and after the heat treatments and ≈0.6 in the OBG solution before and after the heat-treatments without ferrous ions. The values of the slope of 0.5–0.7 indicate that β-glucan molecules remained as random coils or a worm-like molecules during heating (Burchard, 2003), which corresponds to the previous findings that cereal β-glucan occurs as a partially stiff worm-like molecule in water solutions (Böhm & Kulicke, 1999a; Gómez, Navarro, Manzanares, Horta, & Carbonell, 1997).

Interestingly, when OBG was heated with the addition of ferrous ions, the highest molar mass species formed compact structures with a slope value significantly lower than 0.5, as seen in Fig. 2a. These high molar mass species were formed in detectable amounts in the presence of 1 mM $FeSO_4$, as seen in Fig. 1b and further illustrated as RI and light scattering signals in Fig. 2b. In Fig. 2b, the small peak eluted before the main peak had a molar mass in the order of $10^{6.5}$ to 10^7 g/mol, and the molar mass of the main peak was in the order of $10^{4.5}$ g/mol. Since the molar mass of the untreated β-glucan in OBG was in the order of 10^5 to 10^6 g/mol (Fig. 2b), the occurrence of two populations primarily indicates degradation, and the secondary association of these degraded molecules.

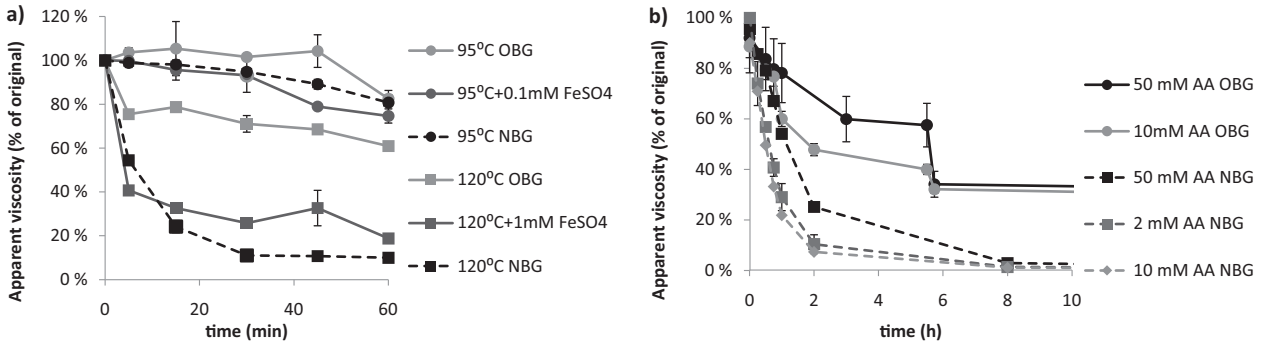

Fig. 3. Decrease of the apparent viscosity of the OBG and NBG solutions (a) after the heat-treatments and (b) during the ascorbic acid treatment.

Bending of the conformation plot, i.e., the high molar mass species with low slope values (Fig. 2a), was obtained after the heat treatment of OBG in the presence of ferrous ions, after the ascorbic acid treatment of OBG, and after the homogenisation of both OBG and NBG. These extremely low slope values approaching zero generally indicate compact cluster formation rather than a hard sphere-like conformation, having a plot value of 0.333 (Burchard, 2003). The occurrence of these compact high molar mass species is consistent with the results of Potthast, Kostic, Schiehser, Kosma and Rosenau (2007), who reported the formation of compact regions in periodate oxidized cellulose, which formed hemiacetal cross-links between the oxidized and unoxidized functional groups. Strlic, Kocar, Kolar, Rychlý and Pihlar (2003) also detected carbonyl group-related cluster formation when heating pullulans. Cluster formation by the hemiacetals between the carbonyl and hydroxyl groups of the degraded chains, having an enhanced mobility (Doublier & Wood, 1995), is a highly plausible explanation for the high molar mass species obtained in the present study. Degraded β-glucan also associates via hydrogen bonds by side-by-side aggregation (Böhm & Kulicke, 1999b), which might have been dissociated by the organic solvent used in the molar mass analysis.

3.5. *Viscosity of β-glucan solutions during heating and ascorbic acid treatments*

Viscosity is generally considered to be the main factor in the health functionality of β-glucan (Wood, 2010), also being an important parameter in food texture, and was thus considered here as a function of treatment time. The viscosity (flow properties) of the aqueous β-glucan solutions was generally influenced by the treatments correspondingly with the molar mass results (Fig. 3a and b). At 95 °C the viscosity moderately decreased over time, while at 120 °C the degradation trend indicated rapid degradation reactions, which consume the reagents or propagate further. This characteristic was especially observed for the extract solution and the OBG solution with 1 mM of $FeSO_4$. However, even though the degree of degradation significantly increased as a function of increasing temperature, β-glucan appeared to be more resistant to thermal degradation than another linear water-soluble polysaccharide, guar galactomannan. The viscosity of the galactomannan solution was reported to decrease by approximately two orders of magnitude when it was heated for 60 min at 100–120 °C (Kök et al., 1999), while the viscosity of NBG decreased by only one order of magnitude over 60 min, despite the two solutions having similar starting viscosities. The relatively highly resistant nature of oat β-glucan in thermal treatment was also observed by Autio, Myllymäki & Mälkki (1987), who reported a D-value (decrease in viscosity to a tenth of the original level) of 145 h when heated at 100 °C. However, in the present study, the D-value was calculated (Fig. 3a) to be 5–10 h at 95 °C for both the OBG and NBG solutions.

As expected, the ascorbic acid-induced viscosity loss was significant in every sample (Fig. 3b), and consistent with the results from previous studies on other water-soluble polysaccharides (Crescenzi et al., 1997; Fry, 1998; Zoldners et al., 2005). Similarly to the molar mass decrease, the order of magnitude of the viscosity loss was 10 mM < (2 mM) < 50 mM. This supports the other indications of the occurrence of a cross-over effect between the concentrations of 10 mM and 50 mM in the studied water-solution conditions. In addition, a more intensive change in the flow properties was obtained in the extract solutions, which supports the promoting role of the co-extracted compounds and highlights the sensitivity of β-glucan in aqueous phases of foods.

4. Conclusions

The present study demonstrated for first time the formation of oxidized functional groups (carbonyls) simultaneously with the chain cleavage of cereal β-glucan. In addition, the organic solvent DMAc was successfully used in β-glucan analysis, which could be useful in further studies on β-glucan degradation and aggregation.

Oat β-glucan was oxidized when its water solutions were treated by ascorbic acid, heating or high pressure homogenisation, seen as the formation of the oxidized functional groups, i.e., carbonyl groups along the β-glucan chain during these processes. Oxidative cleavage may thus explain the obtained decrease in the molar mass of β-glucan simultaneously with carbonyl group formation. Thermal and ascorbic acid-induced oxidation was more significant in the solution of native β-glucan with the co-extracted compounds, and no clear evidence of oxidation in the thermal treatment of highly purified β-glucan without added ferrous ions was actually obtained. In presence of the ferrous ions, the heat-treatments at 120 °C resulted in significant contents of carbonyl groups, similarly to the ascorbic acid-treated samples. Furthermore, thermal treatment-induced oxidation was observed to progress, since the carbonyl content decreased as a function of increasing exposure time. One of the progressive reactions was obtained in the study by the formation of highly compact high molar mass species of ascorbic acid-treated, heat-treated and high pressure homogenised purified β-glucan. The highly compact structures were most likely clusters formed by hemiacetal cross-links via oxidized and unoxidized functional groups. Unlike after the other treatments, the compact high molar mass species were also formed in the extract solution with co-extracted compounds after high pressure homogenisation. This may be due to the intense degradation of β-glucan in the homogenisation treatment. The degradation of β-glucan was most probably predominantly caused by shear and elongational stress, but oxidation may have had a role by affecting the molecular properties of the homogenised β-glucan, as shown by the formation of carbonyl groups along the β-glucan chain. This study demonstrated for first time that the β-

glucan chain was oxidized in treatment processes, posing a risk to the functionality of β-glucan in solutions. On the other hand, the fragmentation and new properties of oxidized β-glucan may prove to be an advantage in utilising β-glucan-rich materials.

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