NON-ENZYMATIC DEGRADATION OF (1→3)(1→4)-β-D-GLUCAN IN AQUEOUS PROCESSING OF OATS

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ACADEMIC DISSERTATION

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ABSTRACT

Cereal water-soluble β-glucan [(1→3)(1→4)-β-D-glucan] has well-evidenced health benefits and it contributes to the texture properties of foods. These functions are characteristically dependent on the excellent viscosity forming ability of this cell wall polysaccharide. The viscosity is affected by the molar mass, solubility and conformation of β-glucan molecule, which are further known to be altered during food processing. This study focused on demonstrating the degradation of β-glucan in water solutions following the addition of ascorbic acid, during heat treatments or high pressure homogenisation. Furthermore, the motivation of this study was in the non-enzymatic degradation mechanisms, particularly in oxidative cleavage via hydroxyl radicals.

The addition of ascorbic acid at food-related concentrations (2-50 mM), autoclaving (120°C) treatments, and high pressure homogenisation (300-1000 bar) considerably cleaved the β-glucan chains, determined as a steep decrease in the viscosity of β-glucan solutions and decrease in the molar mass of β-glucan. The cleavage was more intense in a solution of native β-glucan with co-extracted compounds than in a solution of highly purified β-glucan. Despite the clear and immediate process-related degradation, β-glucan was less sensitive to these treatments compared to other water-soluble polysaccharides previously reported in the literature. In particular, the highly purified β-glucan was relatively resistant to the autoclaving treatments without the addition of ferrous ions.

The formation of highly oxidative free radicals was detected at the elevated temperatures, and the formation was considerably accelerated by added ferrous ions. Also ascorbic acid pronounced the formation of these oxidative radicals, and oxygen was simultaneously consumed by ascorbic acid addition and by heating the β-glucan solutions. These results demonstrated the occurrence of oxidative reactions, most likely the metal catalysed Fenton-like reactions, in the β-glucan solutions during these processes. Furthermore, oxidized functional groups (carbonyls) were formed along the β-glucan chain by the treatments, including high pressure homogenisation, evidencing the oxidation of β-glucan by these treatments. The degradative forces acting on the particles in the high pressure homogenisation are generally considered to be the mechanical shear, but as shown here, carbohydrates are also easily degraded during the process, and oxidation may have a role in the modification of polysaccharides by this technique.

In the present study, oat β-glucan was demonstrated to be susceptible to degradation during aqueous processing by non-enzymatic degradation mechanisms. Oxidation was for the first time shown to be a highly relevant degradation mechanism of β-glucan in food processing.
ACKNOWLEDGEMENTS

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CONTENTS

ABSTRACT .......................................................................................................................... 3

ACKNOWLEDGEMENTS ................................................................................................. 4

LIST OF ORIGINAL PUBLICATIONS .............................................................................. 8

ABBREVIATIONS ............................................................................................................. 9

1 INTRODUCTION ........................................................................................................ 10

2 REVIEW OF THE LITERATURE .............................................................................. 12
  2.1 (1→3)(1→4)-β-D-Glucan .................................................................................. 12
     2.1.1 Molecular properties ............................................................................... 12
     2.1.2 Aggregation behaviour .......................................................................... 14
     2.1.3 Flow properties of oat β-glucan ............................................................ 16
     2.1.4 Health functionality of oat β-glucan ..................................................... 17
  2.2 Degradation of oat β-glucan in aqueous processes ......................................... 19
     2.2.1 Baking ................................................................................................... 19
     2.2.2 Freezing ............................................................................................... 20
     2.2.3 Processes with mechanical energy input .............................................. 20
  2.3 Degradation Mechanisms of soluble polysaccharides .................................. 22
     2.3.1 Oxidative cleavage ............................................................................... 22
           2.3.1.1 Metal catalysed hydroxyl radical production ................................ 25
           2.3.1.2 Ascorbic acid and oxidation ....................................................... 26
     2.3.2 Thermal degradation ............................................................................ 28
           2.3.2.1 Acid hydrolysis ........................................................................... 29
           2.3.2.2 Degradation under alkaline conditions ....................................... 30
     2.3.3 Hydrolysis by mechanical energy ....................................................... 31
  3 AIMS OF THE STUDY ............................................................................................ 34

4 MATERIALS AND METHODS ............................................................................. 35
  4.1 Materials and sample preparation .................................................................... 35
  4.2 Treatments and processes (I-V) ........................................................................ 35
4.3 Methods................................................................................................... 36
4.4 Characterisation of the solutions (II - V).................................................... 36
4.4.1 Analysis of degradation (I-V).................................................................. 37
4.4.2 Analysis of oxidation (III, V)................................................................. 39
4.4.3 Illustration of aggregates and assemblies of β-glucan......................... 41
5 RESULTS....................................................................................................... 42
5.1 Chemical composition of the solutions (I-V).............................................. 42
5.2 Ascorbic acid induced degradation of β-glucan (I,II,III,V)....................... 43
5.2.1 Molecular changes of β-glucan in ascorbic acid treatments (III, V)........ 45
5.2.2 Oxidative reactions in solutions containing ascorbic acid (I,II,IV)......... 46
5.2.3 Carbonyl group formation in ascorbic acid treatments (V).................... 47
5.3 Thermal degradation of β-glucan (III)........................................................ 48
5.3.1 Oxidative reactions in the thermal treatments (III)............................... 49
5.3.2 Carbonyl group formation during heating (V) ...................................... 50
5.4 Degradation of β–glucan in Homogenisation (IV,V)................................. 51
5.5 Aggregation of β-glucan.......................................................................... 53
6 DISCUSSION.................................................................................................. 55
6.1 Ascorbic acid induced oxidation of β-glucan ........................................... 55
6.2 Thermal oxidation of β-glucan ................................................................. 58
6.3 Homogenisation induced degradation of β-glucan................................. 60
6.4 Effect of the co-extracted compounds on oxidation ............................... 61
6.5 Cross-linking of treated β-glucan ............................................................. 63
6.6 Aspects of the management of β-glucan in liquid foods ......................... 63
6.7 The aggregation phenomenon in the study ............................................. 65
7 CONCLUSIONS ............................................................................................ 66
REFERENCES ........................................................................................................ 68
LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are referred in the text by their Roman numerals:


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Contribution of the author to papers I-V:

Reetta Kivelä had the main responsibility for planning the studies and managing the experiments. The HPSEC-, LS- and ESR-methods were used under the guidance of other authors. Reetta Kivelä interpreted the results together with the other authors, and acted as the corresponding author of the papers.
ABBREVIATIONS

β-glucan  (1→3)(1→4)-β-D-glucan

Critical concentrations

c*  Critical concentrations

c[n]  Coil overlap parameter

Degree of polymerisation

Degree of aggregation

Degree of substitution

Degradation rate

Mark Houwink -parameter

Weight average molecular weight

Original weight average molecular weight/M$_w$ at time $t$

Peak molecular weight

Number average molecular weight

Radius of gyration

Radius of root mean square

Hydrodynamic radius

Shape parameter

Specific refractive index increment

Zero shear viscosity

Specific viscosity

Apparent viscosity at a specific shear rate

Intrinsic viscosity

Elastic modulus (mechanical properties of a solution)

Viscous modulus (mechanical properties of a solution)

Organic solvent dimethylacetamide/lithiumchloride

High performance size exclusion chromatography

Light scattering

Multi-angle laser light scattering

Right-angle laser light scattering

Low-angle laser light scattering

Dual-angle laser light scattering

Dynamic light scattering

High performance anion exchange chromatography

Pulsed amperometric detection

High performance liquid chromatography

Electron spin resonance spectroscopy

Ferrous-xylanol orange method

Microfluidizer high pressure homogeniser

High pressure valve homogeniser

Carboxymethyl cellulose

Low-density and very low-density lipoproteins

Solution of native oat β-glucan with low purify. degree

Solution of highly purified acid hydrolysed oat β-glucan
Cereal soluble fibre, (1→3)(1→4)-β-D-glucan, has gained a considerable attention among researchers, consumers and the food industry since the 1980s when its bioactivity was discovered. The scientifically evidenced health benefits of this β-glucan have been acknowledged by several authorities. The US Food and Drug administration FDA allowed a generic health claim ‘may reduce the risk of heart disease’ (FDA 1997) in 1997 including a requirement of a minimum level of 0.75g of soluble beta-glucan fibre per serving and 3 g per day. After that, several authorities in Europe have claimed or pro-claimed the cholesterol lowering ability of β-glucan [Swedish Code of Practice, 2001 and 2006; JHCl (UK), 2005; AFFSA (France), 2008; EFSA(EU), 2009; EFSA (EU), 2010]. Recently, the European Food and Safety Administration EFSA gave two positive opinions considering oat β-glucan. The opinion ‘Regular consumption of beta-glucans contributes to maintenance of normal blood cholesterol concentrations’ was given in 2009 and limited to unprocessed and minimally processed oat and barley products and 3 grams daily consumption of β-glucan (EFSA, 2009). In 2010 EFSA worded that ‘Oat beta-glucan has been shown to lower/reduce blood cholesterol. Blood cholesterol lowering may reduce the risk of (coronary) heart disease’ when consumed 3 grams of β-glucan per day in a balanced diet (EFSA, 2010). Another highlighted health function of β-glucan is its ability to attenuate serum glucose and insulin response after meals, which was positively recognized by the Swedish Code of Practice in 2002.

The health claims have led to development of fractionation methods for β-glucan -rich milling products, and furthermore expanded the use of oat from flakes and porridge to a wide range of products. However, most of the claims and official positive opinions of β-glucan are fixed not only to the concentration, but also to the minimally processed foods to ensure the quality of the consumed β-glucan. The chain of β-glucan may degrade in processing (Åman et al., 2004), which affects the solution properties and possible the health properties of β-glucan (Tosh et al., 2010). The degradation mechanisms of β-glucan, especially when isolated in aqueous environment, are poorly reported, which creates challenges for determination of the health benefits and development of β-glucan enriched products.

The present study focused on elaborating the non-enzymatic degradation trends of oat beta-glucan during the processing of its water-solutions. Previously, acid hydrolysis has been exclusively used to explain the non-enzymatic, process-related degradation of β-glucan, and other chemical reactions have been neglected. In the beginning of the present work, polysaccharide oxidation turned out to be a potential degradation mechanism of β-glucan, and based on the findings on the degradative effect of ascorbic acid communicated in the study I, the present study concentrated on oxidation to explain the food process-related
degradation of oat β-glucan. Scientists working with cellulose have shown the importance of oxidation reactions in damaging cellulose fibres, but also as desired modifiers of the polysaccharide (Potthast et al., 2006). In addition, oxidation has been demonstrated to be an important non-enzymatic degradation mechanism of cell wall polysaccharides during the plant growing phase (Miller, 1986; Vreeburg and Fry, 2005). In the wide frame, the present work aimed to extend the toolbox of product developers and nutritionists to control β-glucan degradation, thereby tailoring the technological properties and the health functions of β-glucan.
Review of the literature

2  REVIEW OF THE LITERATURE

2.1  (1→3)(1→4)-β-D-GLUCAN

β-Glucan typically comprises 3.5 – 5.0 % of the dry matter of whole oat grain. The content is affected by several factors such as the cultivar and growing conditions (Asp et al., 1992). β-Glucan is distributed throughout the starchy endosperm in the cell walls, but is concentrated in the bran (aleurone and sub-aleurone layer) fractions of the oat grain (Fig. 1). Oat bran fractions roughly comprise 6-8%, oat bran concentrates 15-20% and β-glucan isolates up to 80% of β-glucan. Oat β-glucan has an excellent water-binding and viscosity forming capacity compared to other soluble cereal fibres (Wood, 1993). These properties may positively contribute the health functions of β-glucan, but present challenges in processing and product engineering.

Figure 1. A) Distribution of β-glucan in the cell walls and bran layers of an oat kernel (adapted from www.oatsandhealth.org by courtesy of VTT Technical Research Centre of Finland). B) Confocal scanning laser microscopy image of an oat bran particle from porridge showing the β-glucan in the cell walls in blue. The image size is 85 μm x 85 μm (unpublished material/Kivelä UH).

2.1.1  Molecular properties

Oat β-glucan is a linear polysaccharide that consists of β-D-glucopyranosyl units. The units are joined by either (1→3)- or (1→4)-β-D-linkages, hence the name mixed-linkage (1→3,1→4)-β-D-β-glucan (Scheme 1). The (1→4)-β-linkages dominate and mostly occur in groups of two or three, forming segments with three and four glucose residues (DP3/cellotrioses and DP4/cellotetraoses). These cellulose-like sequences are interrupted by separated (1→3)-β-linkages as first demonstrated by Parrish et al. (1960) and later by others. The (1→3)-β-linkages increase the flexibility of the chain (Buliga et al., 1986) preventing the close packing known for cellulose molecules, and enabling the water-solubility of cereal β-glucan. The ratio of (1→4)-β- to (1→3)-β-linkages is approximately
Review of the literature

70:30, and the main building blocks cellotrioses and cellotetraoses comprise over 90% of the molecule. A minor part of the oat β-glucan structure consists of longer cellulose-like sequences up to DP20, of which DP5, DP6 and DP9 are the most abundant, the ratios varying between cultivars (Doublier and Wood, 1995; Lazaridou et al., 2003, Fincher et al., 2009).

Scheme 1. Schematic structure of the (1→3)(1→4)-β-D-glucan, and the building block cellotriose.

The weight average molar mass ($M_w$, or peak molar mass $M_p$) of oat β-glucan is typically 1000-3000 x 10^3 g/mol (Lazaridou and Biliaderis, 2007a). For example, the molar masses of β-glucan in oat whole flour, rolled oats, oat bran and oat bran concentrates were between 2060-2300 x 10^3 g/mol, determined as relative weight average molar masses (HPSEC-fluorescence) after aqueous extraction by amylases and proteases (Åman et al., 2004). Wood (1991) reported molar masses of 2900-3100 x 10^3 g/mol from oat groat and bran after alkaline extraction of various cultivars. The discrepancies in the molar mass results are mainly derived from the analysis and extraction methods, and less from the grain fraction or the cultivar. In addition, ethanol precipitation, widely used for the purification of the polysaccharides, decreased the molar mass from ≈3000 000 g/mol to 2100 000 g/mol and an (NH₄)₂SO₄ -purification step to 400 000 g/mol (Wood, 1991).

Figure 2. The apparent conformation of dissolved wheat β-glucan with molar mass of 53 672 g/mol as a three dimensional computer modelled illustration (adapted with the permission of Springer from Li et al., 2011b).

In water solutions, the conformation of the single-stranded β-glucan is a partially stiff worm-like molecule (Fig. 2) and an expanded coil having Mark-Houwink’s exponent of 0.6-0.7 in the order oat > barley > wheat (Table 1; Gomez et al., 1997b; Böhm and Kulicke, 1999a; Wu et al., 2006; Li et al., 2011a). The exponent $\alpha$ of the Mark-Houwink equation $[\eta] = K M_\alpha^\alpha$ is in the range of 0.5 ≤ $\alpha$ ≤ 0.8 for flexible molecules and random coils. The Mark-Houwink equation, where K is a polymer-specific constant, relates the volume occupied by a molecule in solution...
Review of the literature

(intrinsic viscosity $[\eta]$) to the molar mass and conformation of the molecule. The coil expansion of oat $\beta$-glucan is probably due to the partial stiffness, which may be caused by the rigid structures of the 1,4-$\beta$-linked segments.

Table 1. Molecular parameters of oat $\beta$-glucan in aqueous solutions. Selected molar masses ($M_w$ or $M_p$), the Mark Houwink exponent $\alpha$, intrinsic viscosity $[\eta]$, value of coil overlap parameter $[\eta]_c$ in critical concentrations $c^*$ and $c^{**}$, aggregation degree $x$ (size, aggregate in water / size, unimer) and the ratio of cellotrioses to tetraoses ($DP3/DP4$) are presented.

<table>
<thead>
<tr>
<th>$M_w \times 10^3$ (g/mol)</th>
<th>$\alpha$</th>
<th>$[\eta]$ (dl/g)</th>
<th>$c^* [\eta]$ (g/dl)</th>
<th>$c^{**} [\eta]$ (g/dl)</th>
<th>$x$</th>
<th>extract. solvent</th>
<th>Analysis method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1160$^a$</td>
<td>2.2</td>
<td>2.4</td>
<td>7.8</td>
<td>pH10.</td>
<td>SEC/Fl</td>
<td>Cui et al., 2000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1190</td>
<td>8.3</td>
<td>2.4</td>
<td>7.8</td>
<td>pH10.</td>
<td>SEC/triple</td>
<td>Tosh et al., 2004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>780</td>
<td>5.6</td>
<td>2.0 1.2 7.8</td>
<td>pH10.</td>
<td>SEC/MALLS/RI</td>
<td>Skendi et al., 2004</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1200</td>
<td>2.4 2.4 2.4</td>
<td>pH10.</td>
<td>SEC/LS/Fl</td>
<td>Ren et al., 2004$^b$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1200</td>
<td>9.6</td>
<td>2.4 0.7 2.5</td>
<td>pH10.</td>
<td>SEC/LS/Fl</td>
<td>Doublier, Wood, 1995</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>3.8</td>
<td>2.0 0.8 2.7</td>
<td>pH10.</td>
<td>SEC/RI</td>
<td>Lazaridou et al., 2003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1200</td>
<td>0.57</td>
<td>11.0</td>
<td>pH10.</td>
<td>SEC/LS/Fl</td>
<td>Wang et al., 2001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>456</td>
<td>0.71$^c$</td>
<td>4.6</td>
<td>pH10.</td>
<td>SEC/MALLS/RI</td>
<td>Gomez et al., 1997b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2025</td>
<td>0.58</td>
<td>4.2</td>
<td>4.2 (NH$_4$)$_2$SO$_4$</td>
<td>SLS/DLS</td>
<td>Li et al., 2011a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5400$^{(barley)}$</td>
<td>2.2</td>
<td>30.5</td>
<td>comm.</td>
<td>SLS/DLS</td>
<td>Grimm et al., 1996</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$Mp (peak molar mass instead of weight average molar mass), $^b$Same material as studied in Doublier and Wood, 1995, $^c$The determination mainly included barley $\beta$-glucan samples, one oat $\beta$-glucan was included

2.1.2 Aggregation behaviour

The tendency of $\beta$-glucan to aggregate in water solution creates variation in the molecular properties of $\beta$-glucan. In dilute aqueous solutions (See definition 2.1.3), oat and barley $\beta$-glucans occur as fringed micelle-type aggregates, which grow side-to-side via hydrogen bonding of the cellotriose-sequences (Grimm et al., 1995; Böhm and Kulicke, 1999b; Tosh et al., 2004; Wu et al., 2006). Vårum et al. (1992) observed that only a fraction of the molecules was involved in association to form large stable aggregates, which highlighted the problems of molecular association from the perspective of analytics. Even a very small number of aggregates may lead to inaccurate results in molecular weight determination by light scattering techniques. For this reason, cellulose dissolving solvents such as cadoxen, cuoxam and 0.5 M NaOH have been used to dissociate the aggregates in prior to analysis (Grimm et al., 1995; Li et al., 2006; Li et al., 2011a).

Li et al. (2011a) concluded that the aggregation process was rapid in water, and the size of the aggregates was limited and concentration-dependent due to the equilibrium between aggregate dissociation and association. They found the degree of aggregation ($x = R_{h, aggregate in water} / R_{h, unimer}$, where unimer is a single $\beta$-glucan molecule) to be lower for the more rigid $\beta$-glucans in the order of wheat
Review of the literature

(x=3.9) < barley (x=4.4) < oat (x=7.5), and thus concluded that the aggregation was controlled by the diffusion rate in dilute solutions. Vårum et al. (1992) determined a degree of aggregation (x = M_w, unimer/M_w, aggregate in water) 2-4 for oat β-glucan and Grimm et al. (1995) 17-70 for barley β-glucan. In contrast, Böhmm and Kulicke (1999a) reported that the aggregation of barley β-glucan was negligible in dilute solutions. The discrepancies may be explained by similar flexibility of the aggregates with aggregation degree of up to x=5, when the aggregation may not be recognized (Grimm et al., 1995). Li et al. (2006) investigated the aggregation of wheat β-glucan by light scattering techniques and demonstrated that β-glucan was aggregated to a negligible degree after HPSEC-fractionation. This dissociating effect of HPSEC-conditions, probably flow and dilution, was previously suggested for oat (Vårump and Smidsrod, 1988) and barley β-glucan (Gomez et al., 1997a). However, Wang et al. (2002) stated that aggregates of oat β-glucan were detected also after the HPSEC-fractionation.

The molar ratio of cellotrioses and cellotetraoses (DP3/DP4) is a characteristic structural indicator of cereal β-glucans, which follows the order of wheat (4.2–4.5) > barley (2.8–3.3) > oat (2.0–2.4, Table 1). This ratio is related to solubility, which follows in order wheat < barley < oat (Cui et al., 2000) and aggregation of β-glucan in a concentrated solution (Böhmm and Kulicke, 1999a and 1999b; Tosh et al., 2004). Thus, contrary to the diffusion-limited aggregation in dilute solutions (Li et al., 2011a), oat β-glucan with a lower cellotriose content had a lower tendency for spontaneous hydrogen bond association i.e. gelation intensity in concentrated solutions (Böhmm and Kulicke, 1999b). In addition to the fine structure, molar mass affects the gelling intensity, since low molar mass β-glucan has a higher tendency for gelation than molecules with high molar mass (Lazaridou et al., 2003; Tosh et al., 2004). This has been explained by the higher mobility of the shorter chains (Doublier and Wood, 1995). Oat β-glucan with a molar mass of approximately 50 000 g/mol gelled (G''=G') in under 2 hours in a 10% solution, while the gelation time was over 40 hours for a β-glucan with a molar mass of approximately 150 000 g/mol (Lazaridou et al., 2003). Furthermore, gels of the lower molar mass β-glucan were weaker than those with higher molar mass when determined by dynamic oscillation, but stronger and less brittle when determined by static compression. β-Glucan gels typically melt at 55-65 °C, while freeze-thaw cycles promote network formation (“cryogelation”). Even relatively dilute (1-4%) solutions formed cross-linked, strong gels after freezing and thawing (Lazaridou and Biliaderis, 2004). In foods, aggregation and gelation are affected by the matrix, interactions with other macromolecules and solutes. For example, the addition of various monosaccharides significantly slowed down the gelation and weakened the cryogels, but the addition of polyols promoted the gelation of high molar mass β-glucan and inhibited the network formation of low molar mass β-glucan (Lazaridou and Biliaderis, 2007b).
2.1.3 Flow properties of oat β-glucan

Due to the water solubility, high molar mass and conformation, cereal β-glucan easily forms highly viscous solutions. The flow properties of a polymer solution depend on the concentration, molar mass and conformation of the polymer, usually illustrated by the term $c[n]$ (coil overlap parameter or reduced concentration), which comprises all the named parameters and indicates the volume that the polymers occupy in the solution. When the coil overlap parameter is plotted (log-log) against the zero shear viscosity, generalized information of the shear viscosity properties of the polymer is attained. The plot of oat β-glucan solutions gives two (Ren et al, 2003) or three (Doublier and Wood, 1995; Lazaridou et al., 2003; Skendi et al., 2003) linear regions. The regions are for dilute, semi-dilute and concentrated solutions, and the points separating them are the critical concentrations $c^*$ and $c^{**}$. The critical concentration $c^*$ between dilute and semi-dilute solution represents the state when the molecules begin to interact with each other in the solution, and the second critical concentration $c^{**}$ between the semi-dilute and concentrated region represents the beginning of entanglements. In the concentrated region, the viscosity increases more intensively with increasing concentration. The relationships $\eta_{sp} = c[n]$, $\eta_{sp} \propto c[n]^{1.6-1.8}$ and $\eta_{sp} \propto c[n]^{3.9}$ have been determined in the dilute, semi-dilute and concentrated regions, respectively, of oat β-glucan solutions (Doublier and Wood, 1995; Lazaridou et al., 2003). Doublier and Wood (1995) first reported critical concentrations for native oat β-glucan ($M_w \approx 1 000 000–1 500 000$ g/mol) to be $c^* \approx 0.1\%$ and $c^{**} \approx 0.3\%$ ($c^*[n] = 0.7$ and $c^{**}[n] = 2.5$). Ren et al. (2003) analysed the same samples and found only two regions and one critical concentration in 0.2–0.4% of β-glucan ($c^*[n] = 2–4$) (Table 1). In the concentrated region, the shear viscosity of oat β-glucan is shear thinning, while it is Newtonian in the dilute region. The shear thinning behaviour is due to the decline in the entanglements resulting from the orientation of the chains in the flow. At low shear rates, the polymers have time enough to reform in the time-scale of the measuring system; thus, the viscosity is not shear rate dependent, and is known as the zero rate viscosity.

The viscosity properties of oat β-glucan solutions are easily altered by solution conditions, and also even by extraction methods and milling process (Zhang et al., 1998). Alkaline conditions increased the yield of β-glucan in extraction (Burkus and Temelli, 1998), but decreased the viscosity of β-glucan solutions effectively, although reversibly (Wood et al., 1991; Wu et al., 2006). The alkaline conditions probably eliminate the hydrogen bonded intra-actions, stiffening the conformation, and thus affect the swelling and viscosity properties of the β-glucan molecule (Wu et al., 2006). The β-glucan chain does not in principle carry charges, and Autio et al. (1987) did not find any effect of added salt (NaCl, 0-10%, w/v) on the viscosity of oat β-glucan solutions. However, Ghotra et al. (2007) demonstrated the association of negatively charged phosphorus compounds in a highly purified oat β-glucan chain, and suggested it to affect the solubility. Vårum
et al. (1992) reported the effect on slightly acidic pH-condition on the solution properties of oat β-glucan due to the associated charges of the chain.

2.1.4 Health functionality of oat β-glucan

Dietary fibre is traditionally divided into two major groups: soluble and insoluble fibre. Approximately one-third of the insoluble fibre of oats has been reported to consist of β-glucan, while the majority of the soluble fibre is β-glucan (Manthey et al., 1999). The definitions are, however, more or less from the analytical point of view. Physiologically, the insoluble fibre is basically a bulking component, but soluble fibres may - in addition to the bulking effect - interfere with the absorption of glucose, lipids and cholesterol, for instance, and have a prebiotic function in the colon (Guillon and Champ, 2000). In addition to the cholesterol- and postprandial glucose-lowering effects, cereal β-glucan has been shown to reduce the blood pressure (Keenan et al., 2002) and increase satiety in humans (Beck et al., 2009), control body weight in mice (Bae et al., 2009), and have anti-inflammatory effects in mice (Davis et al., 2004).

In general, the main mechanism for the health benefits of soluble fibres is hypothesised to be their capacity to form viscous solutions, and cereal β-glucan has strong evidence of the same mechanism as recently reviewed by Wood (2010). Increased luminal viscosity in the gut modifies absorption rates, possibly by slowing the mixing of the gastrointestinal contents and reducing diffusion as measured by an increase in the so-called unstirred layer adjacent to the mucosa. This consequently delays the absorption of nutrients such as glucose, lipids and bile acids (Story and Kritchevsky 1976, Vahouny et al., 1980). In clinical studies on humans, an inverse linear relationship has been demonstrated between \( \log(M_w \times c) \) and the postprandial glucose/insulin response or serum cholesterol levels (Wood et al., 2000, Mäkeläinen et al., 2006, Regand et al., 2009 and Wolever et al., 2009). In the factor \( M_w \times c \), \( M_w \) is the weight average molar mass and \( c \) is the concentration of the polysaccharide. The factor is related to shear viscosity by the Mark- Houwink relationship \( ([\eta]) = K (M_w)^\alpha \) (where \( K \) and \( \alpha \) are constants) and the relation of shear and intrinsic viscosities \( (\eta_{sp,0} \propto c[\eta]) \) discussed in section 2.1.3. Thus, the inverse relation of the health function and \( M_w \times c \) demonstrates the importance of the viscosity of β-glucan solutions and molar mass of β-glucan in the health functions of oat β-glucan. The clinical studies that provide the physiological activity of β-glucan, often lack the analysis of the physicochemical properties of the oat products (Salovaara et al., 2007), which creates challenges to define role of viscosity in the health benefits of β-glucan.

The increase of the luminal viscosity by β-glucan is the generally accepted mechanism in the serum glucose and insulin response attenuating ability of oat products. However, the relation between viscosity and the cholesterol lowering effect remains somewhat contradictory. In addition to molar mass and
extractability i.e. viscosity, other molecular characteristics, co-extracted compounds and the food matrix have been suggested to contribute to the level of blood lipids. For example, Kerkchoffs et al. (2003) suggested that the food matrix had a more relevant role than the molar mass of oat β-glucan, since they found no effects on serum cholesterol levels by following the consumption of 5 g of β-glucan per day in the form of yeast-leavened wheat bread enriched with oat-bran, while oat bran consumed as a drink was effective. Frank et al. (2004) concluded that the consumption of wheat bread containing oat bran lowered the hypercholesterolemic factors of female subjects, irrespectively of the molar mass of β-glucan (217 000g/mol and 797 000g/mol) of the consumed bread. However, neither the viscosity after extraction in physiological conditions nor the concentration of β-glucan in the extracted solutions was measured in these studies, which may affect the conclusions. Many studies have demonstrated that the extractability of β-glucan is enhanced as the molar mass decreases. For example, Tosh et al. (2010) recently reported similar digestion viscosities in vitro for oat bran and extruded bran with highly degraded β-glucan, since the extractability increased from 40% to 100% as a result of the molar mass decrease from 2200 000 to 210 000 g/mol. However, the dough and bread matrix is highly complex, and the extractability of β-glucan, even when degrading in the process, is not straightforwardly enhanced during baking (Johansson et al., 2007, Rimsten et al., 2003, Moriartey et al., 2010). Some of the published clinical studies of oat products (oat gum instant whip and bread) have failed to detect any significant reduction in serum cholesterol levels despite of the high molar mass of β-glucan in products (Beer et al., 1995; Törrönen et al 1992), which was suggested to be due to the poor solubility of the β-glucan in the test products used. Björklund et al. (2005) examined the effect of oat and barley beverages containing 5 g and 10 g of β-glucan, and found a significant reduction in total serum cholesterol levels, as well as in insulin and glucose levels only with the oat β-glucan beverage containing the lower amount (5 g) of β-glucan. The molar masses ($M_w$) of β-glucans were low, 70 000 g/mol in oat and 40 000 g/mol in barley β-glucan, although liable to be well solubilised in the liquid product. Önning et al. (1999) found that the consumption of oat milk (0.5g β-glucan/100 ml, 750 ml/day) significantly reduced the total and LDL-cholesterol levels in 5 weeks. Naumann et al. (2006) suggested that molar mass alone does not predict the cholesterol lowering effects of β-glucan, since their drinkable product with β-glucan molar mass of only 80 000 g/mol effectively decreased the serum cholesterol concentrations in their human studies. The role of the gut viscosity in the cholesterol-lowering effect was also refuted also by Immerstrand et al (2010), who suggested that the molar masses and viscosity properties of β-glucan in oat products may not be crucial parameters for their cholesterol-lowering effects, based on their studies on cholesterol sensitive mice feed with oat bran containing a varying molar mass of β-glucan (<10 000 -2350 000 g/mol). Bae et al. (2010) noted that the bile acid binding capacity of β-glucan in rats increased after cellulase hydrolysis of β-glucan, resulting in a decrease in molar mass from 1400 000 g/mol to 700 000 g/mol. According to these studies, no particular value for molar masses efficient in the cholesterol maintenance can currently be set,
and more research is obviously needed to understand the critical properties of β-glucan to actively act in nutrition.

2.2 DEGRADATION OF OAT β-GLUCAN IN AQUEOUS PROCESSES

Oats are traditionally consumed as flakes and porridge, but since the discovery of the health benefits of β-glucan, the use of oat has expanded to many food categories such as wide range of bakery products, healthy snacks and drinkable products. From the technological perspective, the degradation of β-glucan is often favoured by processing to enhance product texture and sensory properties. However, uncontrolled degradation may spoil the product structure and adversely affect the health benefits. The *aqueous process* is defined here as a process that uses water as a major ingredient and β-glucan can solubilise to the aqueous phase during such processing.

2.2.1 Baking

The baking process is one of the most studied aqueous processes of β-glucan enriched oat products. The baking process, namely fermentation and the mixing steps of the oat bran in the presence of water and wheat/rye flours, degrades β-glucan (Degutyte-Fomins et al., 2002; Rimsten et al., 2003; Kerckhoffs et al., 2003; Åman et al., 2004; Andersson et al., 2008; Regand et al., 2009). The molar mass of barley β-glucan was reported to significantly and linearly decreased as the fermentation (0–90 min) and mixing (3–10 min) time of wheat bread doughs increased (Andersson et al., 2004). The rapid degradation of β-glucan resulted in three molar mass populations, merely shifting towards lower molar mass species with increasing fermentation time. When oat and barley concentrates were fermented with lactic acid bacteria, a significant decrease in viscosity was obtained during the fermentation, but no fermentation-induced degradation of β-glucan was detected (Lambo et al., 2005). The amount of β-glucan with a higher molar mass significantly decreased, but not that of the low molar mass β-glucan, when barley β-glucan with different molar masses (640000 g/mol and 210 000 g/mol) was added to wheat bread dough (Cleary et al., 2007). The baking-related degradation was also slowed down by a larger particle size of oat bran (Åman 2004), which may be explained by the lower extractability of the β-glucan from oat bran with a larger particle size (Regand et al., 2009). Beer et al. (1997) reported that the manufacturing of muffin, a widely used model product of yeast-free baking, significantly reduced the molar mass of β-glucan recipe dependently (with and without wheat flour, Table 2). No degradation of β-glucan in wheat flour muffins was detected by others (Åman et al, 2004; Tosh et al., 2008). In addition to the baking processes, other aqueous processes with enzyme-active flours, such as pasta preparation, have also been reported to significantly degrade β-glucan (Åman et al., 2004; Regand et al., 2009).
Within degradation, the solubility of β-glucan has been reported to be enhanced in incubation with rye flour and rye sourdough fermentation (Degutyte-Fomins et al 2002), in the fermentation step (Johansson et al., 2006), in muffin baking (Beer et al., 1997), in oat crisp baking (Regand et al., 2009) and in extrusion (Tosh et al., 2010). However, the extractability/solubility of β-glucan was not enhanced by significant degradation during pasta manufacturing (Regand et al., 2009), and the extractability decreased during the baking of yeast-leavened bread (Johansson et al., 2007). The extractability of β-glucan significantly increased in muffins baked without gluten and wheat flour, but when baked with wheat flour, the extractability remained at the level of the oat bran-ingredient (Beer et al., 1997). The processing of oat porridge by cooking is a gentle process, where the molar mass is not altered, but extractability is enhanced (Åman et al., 2004; Johansson et al., 2007; Regand et al., 2009).

2.2.2 Freezing

The role of frozen storage in the molecular change of β-glucan has been discussed, due to its cryogelation ability. Frozen storage has been reported to decrease the extractability, but not to change the molar mass of β-glucan (Kerkhoffs et al., 2003; Cleary et al., 2007). Lazaridou and Biliaderis (2004) noted that cryogelation of solutions of purified β-glucan slightly decreased the weight average molar mass of high molar mass β-glucan (1700 000-2000 000g/mol), had no effect on intermediate β-glucan s (900 000-1400 000g/mol) and increased the molar mass of low molar mass β-glucans (60 000-160 000g/mol). This illustrates the complexity of the process, including aggregation but not necessarily chain cleavage.

2.2.3 Processes with a mechanical energy input

Extrusion is an example of a food process that combines mechanical and thermal energy. No kinetic study of the effect of different parameters on the physicochemical parameters of β-glucan has been reported, but the technique has been used for fragmentation purposes (Tosh et al., 2010). The extrusion parameters include the temperature, water activity and shear forces, which may all affect the degradation of β-glucan. For example with decreasing the temperature from 237°C to 181°C simultaneously with the mechanical energy from 148kW/h to 135 kW/h and increasing the water content from 7% to 19%, considerable degradation of oat bran β-glucan was avoided (Table 2, Tosh et al. 2010). Similarly, no degradation was obtained, when macaroni and flakes were produced from oat bran by extrusion at 115°C (Åman et al, 2004). A decrease in viscosity and molar mass was obtained during pilot plant extraction of oat gum, the manufacture of which included homogenisation and pumping (Wood et al., 1989). High shear rates (24 000 rpm, 16 000 s⁻¹) solely applied to a β-glucan
solution, caused no permanent viscosity loss (Wood et al., 1989; Vaikousi and Biliaderis, 2005), but sonication clearly reduced the molar mass of β-glucan when barley grains or β-glucan solutions were treated (Izydorczyk et al., 2000; Böhm and Kulicke, 1999a). The manufacture of drinkable oat products usually involves enzymatic treatment, heating steps, but also high pressure homogenisation. Extensive degradation of β-glucan has been reported in processing oats to manufacture drinkable products with an apple juice (Åman et al., 2004) or to milk-like products (Table 2). When β-Glucan with a molar mass of 200 000 g/mol was processed into an oat beverage, the molar mass decreased to ≈100 000 g/mol, whereas the manufacturing process did not affect the molar mass of β-glucan with a molar mass of ≈100 000 g/mol (Naumann et al., 2006; Lazaridou et al., 2007). This suggests the occurrence of limiting molar masses, and polymeric fragmentation of β-glucan during these processes. No data are available on the storage-related degradation of β-glucan in the liquid products.

Table 2. Alteration of the molar mass of β-glucan from $M_{w,0}$ ($\times 10^3$ g/mol) to $M_{w,t}$ ($\times 10^3$ g/mol) during the aqueous processing of oat bran or rolled oats. The note (frozen) is for products, which were frozen before the analysis.

<table>
<thead>
<tr>
<th>Product</th>
<th>Process</th>
<th>$M_{w,0}$</th>
<th>$M_{w,t}$</th>
<th>Parameters</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast cereals</td>
<td>extrusion</td>
<td>2000*</td>
<td>1900</td>
<td></td>
<td>Rimsten et al., 2003</td>
</tr>
<tr>
<td>Breakfast cereals</td>
<td>extrusion</td>
<td>2100</td>
<td>1900</td>
<td>115°C/6MPa+ 300°C</td>
<td>Åman et al., 2004</td>
</tr>
<tr>
<td>Breakfast cereals</td>
<td>extrusion</td>
<td>2500</td>
<td>2200</td>
<td>181°C/135kW/h/19%**</td>
<td>Tosh et al., 2010</td>
</tr>
<tr>
<td>Breakfast cereals</td>
<td>extrusion</td>
<td>2500</td>
<td>530</td>
<td>228°C/145kW/h/15%**</td>
<td>Tosh et al., 2010</td>
</tr>
<tr>
<td>Breakfast cereals</td>
<td>extrusion</td>
<td>2500</td>
<td>210</td>
<td>237°C/148kW/h/7%**</td>
<td>Tosh et al., 2010</td>
</tr>
<tr>
<td>Pasta</td>
<td>extrusion</td>
<td>2100</td>
<td>1900</td>
<td>40°C, 10MPa, boiled</td>
<td>Åman et al., 2004</td>
</tr>
<tr>
<td>Pasta</td>
<td>extrusion</td>
<td>2500</td>
<td>500</td>
<td></td>
<td>Regand et al., 2009</td>
</tr>
<tr>
<td>Fresh pasta</td>
<td></td>
<td>2100</td>
<td>600</td>
<td>boiled (frozen)</td>
<td>Åman et al., 2004</td>
</tr>
<tr>
<td>Bread</td>
<td>baking</td>
<td>2000*</td>
<td>400</td>
<td></td>
<td>Törörnen et al., 1992</td>
</tr>
<tr>
<td>Bread</td>
<td>baking</td>
<td>2700</td>
<td>1300a</td>
<td>(frozen)</td>
<td>Sunberg et al., 1996</td>
</tr>
<tr>
<td>Bread</td>
<td>baking</td>
<td>2000*</td>
<td>600</td>
<td></td>
<td>Rimsten et al., 2003</td>
</tr>
<tr>
<td>Bread</td>
<td>baking</td>
<td>2200</td>
<td>240-1700b</td>
<td>220°C/8 min (frozen)</td>
<td>Åman et al., 2004</td>
</tr>
<tr>
<td>Crisp bread</td>
<td>baking</td>
<td>2000*</td>
<td>900</td>
<td></td>
<td>Rimsten et al., 2003</td>
</tr>
<tr>
<td>Crisp bread</td>
<td>baking</td>
<td>2500</td>
<td>200</td>
<td></td>
<td>Regand et al., 2009</td>
</tr>
<tr>
<td>Muffin</td>
<td>baking</td>
<td>1400-1700</td>
<td>600-1000</td>
<td>200°C/20 min (frozen)</td>
<td>Beer et al., 1997</td>
</tr>
<tr>
<td>Muffin</td>
<td>baking</td>
<td>2100</td>
<td>1900</td>
<td>220°C/8 min (frozen)</td>
<td>Åman et al., 2004</td>
</tr>
<tr>
<td>Muffin</td>
<td>baking</td>
<td>2500</td>
<td>2200</td>
<td>180°C/30-60 min</td>
<td>Tosh et al., 2008</td>
</tr>
<tr>
<td>Muffin</td>
<td>baking</td>
<td>600</td>
<td>400</td>
<td>205°C/12 min</td>
<td>Kim and White, 2011</td>
</tr>
<tr>
<td>Apple juice</td>
<td>homog. past.</td>
<td>2100</td>
<td>600</td>
<td>95°C/5 min</td>
<td>Åman et al., 2004</td>
</tr>
<tr>
<td>Porridge</td>
<td>boiling</td>
<td>2500</td>
<td>1900</td>
<td></td>
<td>Regand et al., 2009</td>
</tr>
<tr>
<td>Pancake batter</td>
<td>enzymatic</td>
<td>2000*</td>
<td>200</td>
<td></td>
<td>Rimsten et al., 2003</td>
</tr>
<tr>
<td>Drink</td>
<td>enz., homog.</td>
<td>2000*</td>
<td>80</td>
<td></td>
<td>Naumann et al., 2006</td>
</tr>
<tr>
<td>Drink powder</td>
<td>enzymatic</td>
<td>2000*</td>
<td>200</td>
<td>Freeze dried</td>
<td>Björklund et al., 2006</td>
</tr>
<tr>
<td>Drink from the powder</td>
<td>enzymatic</td>
<td>200</td>
<td>70</td>
<td></td>
<td>Björklund et al., 2006</td>
</tr>
</tbody>
</table>

* Not reported, estimated from other studies, ** Water content, ^depends on the fermentation t, ^added as β-glucan solution to wheat muffin batter, ^^receipt with milk, no other cereals than oat, ^only oat.
2.3 DEGRADATION MECHANISMS OF SOLUBLE POLYSACCHARIDES

The process-related degradation of β-glucan is based on several mechanisms, the predominance of a mechanism depends on the process type, parameters and conditions. Water as a carrier enables the solubility and mobility of β-glucan, and also its diffusion to enzymes or chemicals able to cleave the chain. Biochemical and chemical reactions may degrade the polysaccharide in solutions containing food additives and enzyme active ingredients. In baking, the ingredients easily bring hydrolytic enzymes and cause the degradation of β-glucan (Andersson et al., 2004; Moriartey et al. 2010), although most of the hydrolytic enzymes of oat are inactivated by the milling processes. In inactivated food systems, the chemical reactions such as oxidation may take place. Thermal and mechanical energy can hydrolyse the polysaccharide chain and sonication is known to degrade polysaccharides via oxidation. Non-enzymatic pathways causing the degradation of β-glucan in solutions, especially the oxidation mechanism and pathways, are focused on here.

2.3.1 Oxidative cleavage

The principle of oxidative processes is commonly the in situ production of free radicals, most often hydroxyl radicals (OH•). These are the most active of the reactive oxygen species, which non-selectively attack a wide range of organic molecules with a reduction potential of >2000 mV (Fig. 3) resulting in the oxidation of the attacked compound. This type of oxidation is in concern in diseases (oxidative stress), and utilised in waste water treatments (Gratzl, 1987) and polysaccharide modification (BeMiller, 2007).

Hydroxyl radicals attack macromolecules in their immediate vicinity in a diffusion dependent manner with rate constants usually in order of 10^6–10^9 M⁻¹s⁻¹ (von Sonntag, 1980; Buxton et al. 1988; Halliwell and Gutteridge, 2007). In the case of carbohydrates, the hydroxyl radicals abstract the hydrogen from C-H moieties of the carbohydrate chain due to the much lower bond dissociation energy of the C-H bond compared to the O-H bond. The formed carbon centered alkoxyl radical, i.e. radicalised polysaccharide chain, relatively easily react with atmospheric oxygen resulting in peroxy radicals (Scheme 2, von Sonntag, 1980; Grazl et al.,...
The α-hydroxalkylperoxyl radicals are able to eliminate the superoxide radical (\(O_2^{•−}\)) and convert to carbonyl groups as shown in Scheme 2 after hydrogen abstraction from C-2. Some of the peroxy radicals, for instance the C-1 and C-4 peroxy radicals involved in the glucosidic bond of cellobiose, may be relatively stable and fragment via oxyl radical formation (Schuchmann and von Sonntag, 1978). The nature of the numerous possible carbonyl species is dependent on which carbon is attacked (von Sonntag, 1980). The carbonyls may further oxidize to carboxyls, and low molar mass products are typically also formed in the oxidation reactions (Potthast et al., 2006).

Scheme 2. A schematic view of carbonyl group introduction by hydroxyl radical (\(•\)OH) attack on C2 of a β-glucan chain (modified from Gratzl, 1987).

The hydroxyl radicals non-selectively attack the carbohydrates, and no pronounced selectivity of glucose carbons on which the hydroxyl radicals attack has been found (Schuchmann and Sonntag, 1978). However, when (1→4)-β-cellobiose was irradiated, 98% of the oxidation products were derived from mechanisms explained by the hydroxyl radical attack on C-1, C-4 and C-5 (Scheme 3, von Sonntag, 1980). In the case of various molar mass (1→6)-α-dextrans, the C-1 involved in the glycosidic bond was reported to be hindered from the hydroxyl radical attack (Gilbert et al., 1984).

Scheme 3. The radicals of cellobiose after the hydroxyl radical attack on C-4, C-1 and C-5, and their possible cleavage mechanisms (modified from von Sonntag, 1980).
The intensity of hydroxyl radical attack is affected by molecular parameters of carbohydrates such as their conformation, crystallisation, association with other compounds and charges of the chain, and reaction conditions such as pH and temperature. For example, CMC with methyl group substitution degree of 1 degraded more readily than CMC with degree of 0.5, due to the negative charges carried by the methyl groups (Crescenzi et al., 1997). The conformation of polysaccharides has been demonstrated to have a significant role in hydroxyl radical induced chain cleavage (Christensen et al., 1996, Hjerde et al., 1998). Despite the similar degree of hydroxyl radical attack on single and triple-stranded glucans, the cleavage of the triple-helix glucan was slow at the beginning, while the single stranded glucan readily degraded (Hjerde et al., 1994). The trend was concluded to be due to a critical conformational loosening, after which the hydroxyl radical attack started to cleave the backbone.

Cellulose has widely been reported to degrade by hydroxyl radical attack (see for example Johansson and Lind, 2005). Considerable alterations in the molar mass have been reported, and furthermore in the functionality of cellulose. Viscosity of aqueous solutions of polysaccharides such as xyloglucan and carboxymethylcellulose (Fry, 1998), scleroglucan (Hjerde, 1998), pullulan (Crescenzi et al., 1997) and xanthan (Christensen et al., 1996) have been demonstrated to considerable decrease by hydroxyl radical attack. This primarily indicates chain cleavage, but also the changes in the molecular properties affecting the viscosity. Furthermore, oxidation may alter the functionality of the polysaccharides by increasing their reactivity to interact with other molecules. This is seen for example as an increasing gel-making ability and enhanced gel properties, as demonstrated by periodate-oxidized scleroglucan (Christensen et al., 2001) and Fenton’s reagent (See 2.3.1.1) oxidized starch (Parovuori et al., 1995). The oxidized polysaccharides may associate by the cross-linking of the carbonyls with the hydroxyl groups of the glucose residues (Potthast et al., 2007), or side-by-side clustering of the radicalized chains (vonSonntag, 1980).

The oxidation of cereal β-glucan was shown to occur as chain scission and carbonyl group formation in the process of cell-wall loosening during the growing phase of plants (Fry et al., 2002). Targeted oxidation of the primary hydroxyl groups (C-6) of oat β-glucan was demonstrated to enhance the cholesterol maintenance properties (Park et al., 2009). The oat β-glucan was oxidized by a stable radical termed TEMPO, and found to enhance the triglyceride, total and LDL/VLDL cholesterol lowering ability of oat β-glucan in hypocholesterolemic rats. This was explained by the enhanced water solubility and in vitro bile acid binding capacity of the oxidized β-glucan due to the acidic carboxy groups formed into the chain. In addition, random oxidation of oat β-glucan by hydrogen peroxide also increased the bile acid binding capacity, although a considerable molecular degradation was detected (de Moura, 2011). Furthermore, the oxidation did not affect the lipid binding capacity, but clearly decreased the gel forming ability. Thus, in addition to degradation, oxidation is a highly interesting mechanism due to the possibility to enhance the interactions of the oxidized β-
glucan chains with the surrounding compounds. However, since the oxidation mechanism and products have almost entirely been neglected in dietary fibre studies, aspects such as the toxicity of the oxidation products, as reported for hydroxyl radical-fragmentated κ-carrageenan (Chen et al., 2011), need to be considered in the future.

2.3.1.1 Metal catalysed hydroxyl radical production

Hydroxyl radicals are in principle produced by homolytic scission of the O-O bond of hydrogen peroxide (H$_2$O$_2$), which may be initiated by metals, heat and radiation, for instance (Halliwell and Gutteridge, 2007). In the industrial modification of carbohydrates, the hydroxyl radicals are produced by numerous pathways such as the reduction of oxygen, hydrogen peroxide and ozone with catalysts such as metals and metaloxides (Arts et al., 1997). Radiation and sonication have also been utilised in the modification of macromolecules (von Sonntag, 1980; Weiss, 2010).

In biological conditions, hydroxyl radical production is most often considered to involve transition metal catalysed hydrogen peroxide reduction (Reaction 1):

$$\text{Fe}^{2+} + \text{H}_2\text{O}_2 + \text{H}^+ \rightarrow \text{Fe}^{3+} + \text{H}_2\text{O} + \cdot \text{OH}$$  \hspace{1cm} (Reaction 1)

The oxidation of organic substrates by iron and hydrogen peroxide is generally called Fenton chemistry (the Fenton reaction or Fenton’s reagent), as it was first described by Fenton in 1894 in an observation of the oxidative capacity of H$_2$O$_2$ and ferrous iron ions (Fenton, 1894). The Reaction 1 was reported by Haber and Weiss (Haber and Weiss, 1934), after observing hydroxyl radical formation from hydrogen peroxide, known as the Haber Weiss cycle (Reactions 2 and 3).

$$\text{H}_2\text{O}_2 + \cdot \text{OH} \rightarrow \text{H}_2\text{O} + \text{O}_2 + \cdot \text{OH}$$ \hspace{1cm} (Reaction 2)

$$\text{H}_2\text{O}_2 + \cdot \text{O}_2^- \rightarrow \text{O}_2 + \text{OH}^- + \cdot \text{OH}$$ \hspace{1cm} (Reaction 3)

The rates of these reactions were later shown to be extremely slow (k is in order of $10^1$ M$^{-1}$ s$^{-1}$) without the presence of metal catalysts, copper being actually more efficient than iron (Buettner and Jurkiewicz, 1996), and the Haber Weiss cycle is often presented as follows:

$$\text{Fe}^{2+} + \cdot \text{O}_2^- \rightarrow \text{Fe}^{3+} + \cdot \text{O}_2^-$$ \hspace{1cm} (Reaction 4)

However, the particular oxidation activity of Fenton chemistry is still under discussion, and the complexity of the process has been highlighted (Koppenol, 2001; Liochev and Fridovich, 2002; Kremer, 2003; Rachmilovich-Calisk et al., 2005, Barbusinski, 2009). The low solubility of iron in biological solutions in the presence of oxygen and its reactivity with several organic matters, as well as the
variable valency of iron, create the complexity in the process. In conditions of pH>1, recent studies have suggested direct bonding between iron and hydrogen peroxide producing metal-peroxo complexes (Reaction 5) alongside Reaction 1 (Kremer, 2003; Rachmilovich-Calís et al., 2005). These high-valent iron-oxo intermediates, most probably ferryl ion complexes \((\text{Fe}^{IV}=\text{O}^2+)\), can proceed to hydroxyl radicals. In addition, the non-radical oxidation i.e. the direct attack of the ferryl compounds on organic matter has been highlighted (Kremer, 2003; Rachmilovich-Calís et al., 2005, Barbusinski, 2009).

\[
\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightleftharpoons \text{Fe}^{IV}=\text{O}^{2+} + \text{H}_2\text{O} \quad \text{(Reaction 5)}
\]

The Reactions 6-8 may also occur in the conditions of Fenton chemistry depending at least on the \(\text{H}_2\text{O}_2/\text{Fe}^{2+}\)-ratio and pH (Halliwell and Gutteridge, 1984, Barbusinski, 2009), and UV-light also affects the reaction pathways. Reaction 8 is a rapid reaction \((k \text{ in order of } 10^8 \text{ M}^{-1}\text{s}^{-1})\), which may terminate hydroxyl radical production especially at low concentrations of hydrogen peroxide.

\[
\begin{align*}
\text{Fe}^{3+} + \text{H}_2\text{O}_2 & \rightarrow \text{Fe}^{2+} + \text{O}_2^- + \text{H}^+ \quad \text{(Reaction 6)} \\
\text{Fe}^{3+} + \text{O}_2^- & \rightarrow \text{Fe}^{2+} + \text{O}_2 \quad \text{(Reaction 7)} \\
\text{Fe}^{2+} + \text{OH}^- & \rightarrow \text{Fe}^{3+} + \text{OH}^- \quad \text{(Reaction 8)}
\end{align*}
\]

In biological systems, iron may also chelate or complex with phenols, proteins and naturally occurring chelates forming a complex with a high reduction potential (Buettner and Jurkiewicz, 1996; Welch et al., 2002). These complexes may be able to reduce dissolved oxygen and produce hydroxyl radicals in aqueous solutions at pH 7 (Welch et al., 2002). It is assumable that not only one universal Fenton mechanism occurs, but both hydroxyl radicals and iron-complexes can coexist in Fenton chemistry and depending on the conditions, one of them will dominate (Rachmilovich-Calís et al., 2005, Barbusinski, 2009). However, the oxidative ability of Fenton chemistry against organic matter is evidenced in highly diversified conditions, and the transition metals, dissolved oxygen and their derivatives are also potential oxidizing agents in foods.

Water-soluble glucans have been demonstrated to be oxidized by Fenton reagents (Parovuori et al., 1995; Hjerde et al., 1998). When plant polysaccharide solutions (CMC, methyl-cellulose, xyloglucan, pectin, alginate and a dextran) were treated with 10 mM hydrogen peroxide in the presence of trace amounts of transition metals at pH 5.5, the viscosity of all the solutions decreased by 20-40% in five hours, indicating chain cleavage of the polysaccharides (Fry, 1998).

### 2.3.1.2 Ascorbic acid and oxidation

The viscosity of several plant polysaccharides has been reported to significantly decrease following the addition of hydrogen peroxide as referred to above, but once the same solutions were treated with both hydrogen peroxide (5 mM) and
Ascorbate (5 mM), or even only with asorbate (10 mM), the viscosity loss considerably accelerated (Fry, 1998). Ascorbic acid, or ascorbate (monoanion) when it occurs in the pH range 4.2-11.6, has long been known to degrade polysaccharides (Robertson et al., 1941; Kertesz, 1943) in neutral or slightly acidic water solutions, and the contribution of hydrogen peroxide to the action of ascorbic acid was demonstrated many years ago (Sumner and Dounce, 1937).

Ascorbic acid is the most common water-soluble antioxidant and food additive, used in juices (antioxidant) and in wheat flours (flour improver). The typical concentration of ascorbic acid in fruit products and juices is 30 mg/100 ml (2 mM), and the daily recommendation in Finland is 60 mg. The antioxidant activity of ascorbic acid is based on the relatively stable ascorbyl radicals (Scheme 4), and their ability to scavenge reactive oxygen species, reactive nitrogen species and non-radical species such as ozone and singlet oxygen (Chou et al., 1983). In addition, ascorbic acid can regenerate other antioxidants such as α-tocopherol (May et al., 1998) and act as a cosubstrate for biologically important enzymes by stabilising the reduced state of transition metals (Levine, 1986).

![Scheme 4. Oxidation of ascorbate from monoanion (AA) to ascorbyl radical (AA•) and dehydroascorbate (DHA).](image)

The ability of ascorbic acid to degrade the water-soluble polysaccharides relates to the excellent reducing ability of ascorbic acid producing hydroxyl radicals via Fenton chemistry (Scheme 4). Ascorbic acid oxidizes in the presence of iron or copper under pH-conditions of 2-8 (Khan and Martell, 1967; Buettner, 1988; Hsieh and Hsieh, 2000). Vice versa, ascorbic acid reduces the metals to act as catalysts in Fenton Chemistry. Ascorbic acid may also be oxidized by molecular oxygen in the presence of trace of transition metals (Buettner and Jurkiewicz, 1996, Guo et al., 2002) and the pro-oxidation may essentially be based on the process shown in Scheme 5 (Guo et al., 2002).

![Scheme 5. The pro-oxidative process of ascorbic acid (ascorbate) including oxidation of ascorbic acid (AA) and the Fenton reaction (Reaction 1, from Guo et al. 2002).](image)
The ascorbic acid induced degradation of xyloglucan was completely inhibited by catalase, and significantly slowed down by ascorbate oxidase and small molar mass compounds such as glucose and mannitol (Fry, 1998). Since the catalase is an enzyme that can decompose hydrogen peroxide, ascorbate oxidase oxidizes ascorbate, and the small molar mass compounds act as competitive free radical scavengers (Morelli et al., 2003), the results support the xyloglucan degradation via the mechanisms illustrated in Scheme 5. The process may be affected by the complexation of metals, oxygen and ascorbate, and the other constituents of biological solutions. In in vivo studies on human cell tissues, the pro-oxidant nature of ascorbate, even in presence of metals, is still highly debated, while pro-oxidative behavior has frequently been demonstrated in vitro (Carr and Frei, 1997).

2.3.2 Thermal degradation

Heating of aqueous materials is commonly used for the pasteurization, sterilization and modification of foods at temperatures of 90-120 °C. Heating influences polysaccharides by accelerating their molecular vibration, collisions and chemical reactions in solutions resulting in side-group elimination, random scission (fragmentation) or depolymerisation of the polysaccharide chain (Pielichowski and Njuguna, 2005). The degradation rate and products depend on the mechanisms, which are altered by the temperature range, processing time, pressure, and solution properties, especially pH. In addition to hydrolysis, oxidation (Robert et al., 2002) and Maillard reactions (Davidek et al., 2006) may contribute to the chain cleavage of polysaccharides at elevated temperatures, which makes the solvent quality an important parameter. The concentration and molecular properties of polysaccharides also affect the intensity of thermal degradation (Bradley et al., 1989).

Lai et al. (2000) pooled thermal degradation data from various studies of common water-soluble polysaccharides, which were heated to 25–160 °C (1% solutions) at pH 6-7 in atmospheric conditions. They found an isokinetic relationship between the different food-related carbohydrates, indicating that they all follow similar reaction mechanism despite the differences in side chains, charges or molecular size. Typically the activation energy values were found to be close to 100 kJ/mol (Lai et al., 2000), which indicates random scission of the polysaccharide backbone (Soldi, 2005). However, the thermal degradation of polymeric material generally follows more than one mechanism (Pielichowski and Njuguna, 2005), and the generalising of the thermal degradation of water-soluble food polysaccharides appears to be somewhat ambiguous. For example, a relatively good thermal stability was observed for CMC compared to galactomannans (locust bean gum and guar gum) and xanthan, as the viscosity of the CMC solution decreased only 1% and over 60% in other solutions during 60 min treatment at 120°C (Mitchell et al., 1991). In addition, locust bean gum and guar gum were reported to significantly differ in their activation energies studied at 70–120 °C(Kök et al., 1999). In the case of oat β-glucan, the backbone was
shown to be cleaved at 120°C, whereas only the degree of aggregation of xyloglucan and dextran was affected under the same conditions (Wang et al., 2001). In another study, the viscosity of oat β-glucan solution was found to be relatively stable against the heating at 100°C when compared to the literature values of CMC (Autio et al., 1987).

2.3.2.1 Acid hydrolysis

Acid hydrolysis is a widely utilised degradation reaction in the fragmentation and modification of polysaccharides such as oat β-glucan (Tosh et al., 2004). Oxonium ions (H₃O⁺) catalyse the hydrolysis of the glycosidic linkages, which is induced at elevated temperatures. The proton of the catalysing acid rapidly interacts with the glycosidic oxygen in the linkage, which is followed by the rapid addition of water-molecules, resulting in the cleavage of the glycosidic bond and stable hydrolysis products.

The rate and products of acid hydrolysis are affected by the type of acid, its concentration, pH, temperature, pressure and also the molecular characteristics of the polysaccharides such as their solubility, structure and conformation. In mild conditions, polysaccharide chains will be randomly cleaved resulting in a decrease in the weight average molar mass. In stronger conditions, hydrolysis results in oligo- and monosaccharides and their derivatives. Typical acid hydrolysis products of glucans in addition to glucose include carboxylic acids such as formic, acetic and lactic acid, and hydroxymethylfurfural (HMF) (widely discussed in the literature on biofuels). Acid hydrolysis of oat β-glucan performed with 0.1 M HCl at 70°C for 30-90 min resulted in fragments with $M_w$-values from 1 200 000 g/mol to 30 000-170 000 g/mol (Tosh et al., 2004). The same acid conditions at 70°C (5h and 12h) resulted in significant amounts of glucose and cellobiose and at 120°C (1h), the recovery of glucose was ≈100% (Johansson et al., 2006). At the biological temperature (37°C) only a slight effect on the flow properties was obtained, and no formation of oligo- or monosaccharides occurred.

Most typically, the acid hydrolysis of water-soluble polysaccharides involves random scission (Wang et al., 2000) similarly to the hydroxyl radical induced cleavage (Christensen et al., 1996). The acid hydrolysis of barley β-glucan ($M_w$=140 000 g/mol) required a certain time at pH 2.5–4.5 before the degradation began (Vaikousi and Biliaderis, 2005), indicating that the hydrolysis did not follow first order kinetics as expected for the water-soluble single-stranded glucan. One explanation for these results may be the aggregation behaviour of the relatively small molar mass β-glucan and thus occurrence of ordered structures. The ordered structures of κ-carragenan and the branched or multiple-stranded water-soluble polysaccharides xanthan and scleroglucan exhibited a similar two-stage acid hydrolysis than reported for barley β-glucan, beginning with a slow-rate stage, and followed by a second stage in which the
apparent degradation rate was much higher (Hjerde et al., 1994; Hjerde et al., 1996; Christenssen et al., 1996).

2.3.2.2 Degradation under alkaline conditions

In foods and food manufacturing, alkaline conditions are rare. In β-glucan analytics, alkaline conditions have been used to enhance the extraction recovery of β-glucan from the food matrices (Dawkins and Nnanna, 1995; Beer et al., 1997; Burkus and Temelli, 1998). Barium hydroxide has been used to enhance the purity and extraction yield of the extracted β-glucan, which may be associated with water-insoluble arabinoxylan (Brummer et al., 2008). In addition, sodium hydroxide has been used to examine the aggregation behaviour of β-glucan due to its ability to dissociate aggregates as demonstrated by light scattering studies (Li et al, 2006).

In an alkaline environment, several degradation reactions start, the most important being peeling and stopping reactions, and alkaline hydrolysis as the hydroxyl ions (OH-) catalyse hydrolysis at elevated temperatures. Peeling reactions start from the existing protonated end group (reducing end) cleaving the glycosidic bonds by β-elimination, i.e. removing the hydroxyl/alkoxyl group in the β-position to the negatively charged oxygen, by one unimer at time (Knill and Kennedy, 2003). Peeling is the dominant reaction resulting in molar mass losses at temperatures <170°C in an alkaline environment, but at temperatures above 170°C alkaline hydrolysis reactions starts to dominate (Nevell, 1985). In hydrolysis, new reducing end groups are formed, which are also new subjects of the peeling reactions. Knill and Kennedy (2003) listed products of the alkaline degradation by several bases of glucose, cellobiose and cellulose within a temperature range of 20-200°C. Organic acids such as formic, acetic, glycolic, lactic and saccharinic acids were formed in significant amounts under all the conditions from all the materials. Similar compounds of organic acids also formed in the alkaline hydrolysis of starch, although at a slower rate (Krochta et al., 1987).

The criticality of alkaline conditions concerns the analysis of processed β-glucan under alkaline conditions. If the β-glucan chain is oxidized in processing, alkaline analysis conditions may alter the information obtained on process-related molar mass-changes. This is because the β-elimination reaction may start not only from the reducing ends, but also from the oxidized functional groups (Potthast et al., 2007; BeMiller, 2007). The carbonyl groups may occur randomly in the chain (Scheme 2), and the β-elimination reaction leads to a steep decrease in the molar mass as shown with cellulose (Fig. 4, Pottast et al., 2006). The effect was obtained under relatively mild alkaline conditions (pH 11/NaOH, 40 °C) with a pre-oxidized cellulose molecule. Figure 4 illustrates the disappearance of the carbonyl groups simultaneously with a molar mass decrease, indicating OH ion induced β-elimination. A similar effect was shown with oxidized xyloglucan, as
the viscosity of its solutions irreversibly decreased by adjusting the pH from 4.7 to 12.7 and back to 4.7 by using NaOH (Miller and Fry, 2001). If covalent linkages had not been cleaved, the neutralisation would have returned the viscosity as also seen with oat β-glucan (Wood et al., 1991).

![Figure 4](image)

**Figure 4.** A) Decrease in the molar mass of pre-oxidized cellulose in alkaline conditions (pH 11, 40°C), B) The simultaneous decrease in carbonyl groups formed in oxidation (adapted with the permission of Springer from Potthast et al., 2006).

The peak molar mass of oat β-glucan decreased from 1900 000 g/mol to 1200 000 g/mol when it was extracted from oat bran with 1.3M NaOH (16 h, 22°C) instead of hot water (2h, 90°C) or under physiological conditions (Beer et al., 1997), indicating the degradation or endwise depolymerisation of β-glucan in alkaline conditions. Alkaline extraction conditions (pH 8-10, 55°C) also resulted in reduced extraction yields of barley β-glucan, which were associated with alkaline depolymerisation (Temelli, 1997; Symons and Brennan, 2004). However, there have been several studies demonstrating successful alkaline extraction, where no degradation or yield losses have been observed. Brummer et al. (2008) reported that oat β-glucan was drastically cleaved by saturated barium hydroxide \([\text{Ba(OH)}_2]\), which is known to affect alkaline hydrolysis of polysaccharides (Knill and Kennedy, 2003). However, the effect of alkalinity in Ba(OH)\(_2\)-derived degradation was excluded in the study. The peak molar mass of oat β-glucan after aqueous extraction (2h, 90°C) was 1 200 000 g/mol, after NaOH extraction (pH 13, 17 h) 1 000 000g/mol and after Ba(OH)\(_2\) extraction (pH 13, 17 h) 70 000 g/mol. The alkaline extraction temperatures were not reported, but barium hydroxide-extraction included an additional heating step (70°C, 3hours, pH 6) to remove the starch by thermo-stable α-amylase, and this step may partially explain the considerably degradation of β-glucan. The authors suggested the degradation to be associated with barium derived oxidation rather than alkaline scission.

### 2.3.3 Hydrolysis by mechanical energy

Mechanical energy is associated with the motion and position of an object. Processes such as pumping, mixing, extrusion and homogenisation apply
mechanical energy on the particles. Isolated and dissolved polysaccharides, in particular, may behave as particles and be affected by the mechanical energy input. Extrusion degraded oat bran β-glucan (Table 2, Tosh et al., 2010) and high pressure homogenisation evidently affects the molecular properties and functionality of solubilised polysaccharides (Yu et al., 1979). For example, microfluidizing was demonstrated to fragmentise chitosan ($M_w,0 \approx 2000\ 000$ g/mol, Kasaaei et al., 2003), xanthan ($M_w,0 \approx 25\ 000$ g/mol, Lagoyete and Paquin, 1998) and gum tragacanth ($M_w,0 \approx 850\ 000$ g/mol, Silvestri and Gabrielson, 1991), and high pressure valve homogenisation degraded water-soluble glucans such as modified cellulose (Floury et al., 2002) and modified starch (Nilsson et al., 2006). The extent of fragmentation is often related to the amount of pressure applied in the system (Stang et al., 2001; Floury et al., 2002).

Homogenisation is widely used in aqueous food applications to enhance product properties such as structure and stability. These are influenced, for instance, by a reduced size and improved dispersibility of particles as a result of mechanical energy that acts on the fluid upon homogenisation. Determination of the precise forces affecting the fluid is challenging, since the dimensions of the force area are small and velocities are high (Innings and Trägårdh, 2007), but elongational and turbulent shear stresses have most often been reported to be the predominant disruptional forces. High pressure valve homogenisers (one or two stage) are classical high pressure homogenisers that are typically used for dairy products. In valve homogenisers, the fluid is pumped through a radial gap and the height of the gap determines the homogenising pressure. In the modern high pressure homogeniser, microfluidizer, the fluid is divided into two micro-channels, which are recombined in a reaction chamber resulting in the collision of the accelerated jets (Paquin, 1999). In these high pressure homogenisers, the disruptional forces are derived from the high flow rate of the fluid in small dimensions, the collisions of the molecules/particles and sudden pressure drop in the nozzle valve/chamber (Walstra and Smulders, 1998; Stang et al., 2001). The type of forces acting on the fluid depends on the properties of both the homogeniser and the fluid, and on the operating parameters (Walstra and Smulders, 1998; Stang et al., 2001). In the case of macromolecular degradation, a limiting molar mass has been identified, i.e. a molar mass under which the molecule is less sensitive to the mechanical energy input (Paquin, 1999). This may explain the observations of β-glucan degradation during the manufacture of drinkable products. The molar mass decreased homogenisation parameter - dependently to $80-100 \times 10^3$ g/mol, and when the original molar mass was close to this, no further degradation occurred during the processing (chapter 2.2.3).

Elongational and turbulent stresses are the dominant degradation mechanism during homogenisation (McClements, 2005), but also hydrodynamic cavitation may also have a role in the disruption of oil droplets by the high pressure homogenisation techniques (Stang et al., 2001). Acoustic cavitation in ultrasonication is widely known to degrade water-soluble polysaccharides (Weiss et al., 2010), which is based on the hydroxyl radical formation by the cavitation
bubbles in the solution (Halliwell and Gutteridge, 2007). The energy of cavitation bubbles can induce shock waves and further homolytic fission of water molecules to hydroxyl and hydrogen radicals, or organic matter to alkoxyl radicals and thus cause oxidation (Halliwell and Gutteridge, 2007). Hydrodynamic cavitation, which occur in high-pressure homogenisers, has been shown to oxidize small molar mass compounds, and high pressure homogenisation has been utilised in the oxidation of waste waters (Gogate and Kabadi, 2009). Lander et al. (2000) demonstrated the contribution of the oxidative reactions in the high pressure homogenisation by the formation of the oxidative free radicals simultaneously with polysaccharide degradation.

The review of literature highlighted that oat β-glucan non-enzymatically degrades during the aqueous processes. However, there is lack of knowledge of the degradation mechanisms and the sensitivity of β-glucan to the potential mechanisms. Oxidative cleavage, and thermal and mechanical energy induced hydrolysis were introduced here as known degradation mechanisms of other water-soluble polysaccharides. In addition, the existing knowledge of the behaviour of β-glucan in the potentially degradative conditions was collected. The present study will focus on studying the potential non-enzymatic degradation mechanisms of β-glucan during aqueous processing, concentrating on the ascorbic acid induced oxidative cleavage, thermal treatments and high pressure homogenisation.
3 AIMS OF THE STUDY

The principal aim of the study was to understand the patterns underlying the degradation of β-glucan during food processing, focusing on the common processes of liquid foods. Oxidation, a previously neglected degradation mechanism, was of particular interest, and mechanical and heat energy input induced degradation was also investigated. The study was performed using solutions of pure oat β-glucan (OBG) and native β-glucan (NBG) with the co-extracted compounds. The aim of using the NBG was to model the reactions of β-glucan in liquid foods and aqueous phases of foods.

The detailed objectives were to:
1. Demonstrate the degradation of β-glucan in ascorbic acid solutions and link the effect to Fenton chemistry and oxidation (I, II, V);
2. Investigate the thermal degradation of β-glucan in solutions and examine the role of oxidation (III, V);
3. Examine the degradation of β-glucan during high-pressure homogenisation (IV) and assess the contribution of oxidation in the microfluidizing treatment (V).
4 MATERIALS AND METHODS

The materials and methods used in the work are briefly described here, and more detailed information is found from the original publications I-V.

4.1 MATERIALS AND SAMPLE PREPARATION

The solution of native oat β-glucan (NBG) was prepared from oat bran concentrate OatWell14% (Swedish Oat Fibre, Våröbacka, Sweden). Oat bran was 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 at 16 000 x g, supernatant was collected and placed in a boiling water bath for 10 minutes to precipitate proteins. After boiling, the dispersions were centrifuged again and the supernatant was used as the sample solution. The NBG is referred to the publication III as OBCet.

The solution of highly purified oat β-glucan (OBG) was prepared from medium viscosity oat (1→3), (1→4) -β-D-glucan (>99 %), supplied by Megazyme International Ltd. The powder was wetted with 99% ethanol (Altia, Finland) before adding the MilliQ water. The dispersion was heated for 3 hours at 80 °C until the solution became clear, and the amount of water was adjusted to obtain a solution with the desired β-glucan concentration. The OBG is referred to in the publication III as MVO.

4.2 TREATMENTS AND PROCESSES (I-V)

L(+)-Ascorbic acid (J.T. Baker, The Netherlands), citric and malic acid (anhydrous, Fluka BioChemika), D (+)-Glucose and D(-)-mannitol (BDH Laboratory Supplies, England), and potassium sulphite (K₂SO₃, Sigma Aldrich Chemie GmbH, Germany) were added to the NBG and OBG solutions mainly as powders at room temperature. Dissolution was ensured by a rigorous mixing for a few minutes. The concentration of 1 mM ascorbic acid was achieved by adding a freshly made 1M solution. The ferrous ions used were added from a fresh 0.1 M solution of iron(II)sulphate heptahydrate (FeSO₄·7H₂O, E. Merck, Germany) to obtain the desired concentrations. Phytic acid was added as a 50% solution (ICN Biomedicals Inc., USA) and catalase (from bovine liver 23 000 U/mg protein, Sigma Aldrich Chemie GmbH, Germany) was added as a solution to produce the desired concentration. When other constituents were used together with ascorbic acid, they were first added to the sample and then followed by the ascorbic acid. All the treatments were performed as aseptically as possible as possible and sodium azide (0.02%) was added to the samples with longer storage.
times (>2 days) to prevent microbial growth. The samples containing catalase were prepared without sodium azide as it may act as a catalase inhibitor.

**Heat treatments (III)** were carried out with OBG and NBG solutions in a dry incubator (95°C) or in an autoclave (120°C) in 15 ml polypropylene-tubes with 5 ml sample for given times. The tubes were carefully sealed, and the absence of evaporation was ensured by weighing the tubes+samples before and after the heat-treatments. Both the temperatures were controlled with an internal sensor. After heating, the samples were allowed to settle down for 3 hours at +6°C prior to viscometric, spectrophotometric and chromatographic analyses.

**Homogenisation treatments (IV)** were carried out with two types of homogenisers, a pilot scale high pressure valve homogeniser (HPVH, Gaulin Laboratory Homogenizer LAB60-10TBS, APV Gaulin GmbH, Holland) and a lab scale high pressure homogeniser (MF, M-110Y Microfluidizer® Processor, USA) with sample amounts of 2 l and 100 ml, respectively. The HPVH treatments were carried out with NBG solutions of 1-2 mg/ml and 3-4 mg/ml of β-glucan using a pressure range of 6–60 MPa (equal to 60-600 bar). MF treatments were carried out with NBG of 1-2mg/ml of β-glucan using a pressure range of 30-100 MPa (equal to 300-1000 bar). The samples for carbonyl group analysis (See section 4.3.3) were prepared from both NBG and OBG by MF with pressures of 30 and 100 MPa. The samples were stored at 6°C for a maximum of 3 hours prior to the viscosity and molar mass analysis. The HPVH treatments were duplicated and the MF-treatments were replicated at least three times.

### 4.3 METHODS

### 4.4 Characterisation of the solutions (II - V)

**The quantification of β-glucan (II- V)** in NBG was performed with an enzymatic method (Approved Method 32-23, AACC 2000) using a Megazyme kit BBG (Megazyme, International Ltd, Ireland). The dry weight content was measured according to Approved Method 44-60 (AACC 2000) with minor modifications (Degutyte-Fomins et al., 2002). The quantification was performed in triplicate from NBG of all series prior to sample preparation.

**The composition and quantity of monosaccharides** (III, glucose, xylose, mannose, arabinose, fructose and galactose) and the quantity of total carbohydrates were analysed with the high performance anion exchange chromatography with a pulsed amperometric detection (HPAEC-PAD) system equipped with two HPLC pumps (Waters 515) and autosampler (Waters 2707) and an electrochemical detector (Waters 2465). Empower software (Waters Corporation, Milford, MA, USA) was used for instrument control and data handling. The analytical CarboPac PA-1 with the quard column (Dionex
Corporation, Sunnyvale, CA) was maintained at 30°C using a flow rate of 1 ml/min with gradient analysis using H₂O and 100mM NaOH as eluents. Prior to the HPLC-analysis, the carbohydrates of extracts were hydrolysed with 4% sulphuric acid into monosaccharides by one hour of 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 applied based on the loss of pure monosaccharides during the acid hydrolysis.

The protein content (II, III, V) of the solutions was analysed from lyophilized extracts and from the powder of pure β-glucan in triplicate using the Dumas combustion method (Vario MAX CN Elementar Analyser systeme GmbH, Germany) and a nitrogen factor of 6.25.

The mineral content (II, III, V) was analysed from lyophilized extracts and from the powder of pure β-glucan after wet-digestion (HNO₃) using inductively coupled plasma-mass spectrometer (ICP-MS, Perkin -Elmer Elan 6000, USA) with an external standard method.

The fatty acid content (III) was determined from NBG by extracting lipids with acidic diethyl ether with an 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).

The β-glucanase activity of NBG (II-V) was determined in a β-glucan-agar-matrix by treatment with congo-red dye, which stains high molecular mass β-glucan. The matrix was prepared from a commercial β-glucan (high viscosity barley, Megazyme International) with a concentration of 0.7% (w/v). Agar (2%, w/v) was dissolved in the β-glucan solution, the system was brought to boil and then heated at 50 °C for 10 minutes. Fresh or 2 or 4 weeks old extract, purified water (negative control) and licchenase solution (50 U/ml, positive control) was added to the cooled gel-matrix. After reacting for 24 hours at 21°C, the samples were rinsed from the matrix and congo-red solution (1% w/v) was pipetted on. After one hour, the dye was rinsed out and the β-glucanase activity of the samples was visualised comparing to the colour intensity with the negative (no colour depletion) and positive (bleached red colour) control.

4.4.1 Analysis of degradation (I-V)

The viscosity (I-V) of the treated solutions was measured with a ThermoHaake RheoStress 600 rheometer (Thermo Electron GmbH, Germany). 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 at 10°C or at 21°C. The samples were measured at given time points, and apparent viscosities at a shear rate of 10s⁻¹ [η_app(10s⁻¹)] were used to
compare the effects of the treatments. The analysis was repeated 3-5 times. The equipment was controlled with standard oils during the study and the method-related variation was determined to be ±15 mPas.

The weight average molar mass ($M_w$), radius of gyration/root mean square ($R_g$ or $R_{RMS}$), hydrodynamic radius ($R_h$), Mark-Houwink’s parameter ($\alpha$) and other polymeric parameters were analysed using high performance size exclusion chromatography (HPSEC) –systems (Table 3). In the technique used in publications I and II, the calibration was based on the commercial β-glucan standard (Megazyme, medium viscosity) and the calibration curve for the molar mass determinations was based on a β-glucan standards of known molar mass, determined by dual angle laser light scattering (DALLS, Suortti, 1993)). In the methods of III and IV, the molar masses were calculated based on the right angle and low angle laser light scattering (RALLS+LALLS=DALLS)/viscometry method using a $dn/dc$ value of 0.151 ml/g (Gomez et al., 1997a). In the publication V, the molar masses were calculated based on the multi-angle laser light scattering (MALLS, 18 angles) using $dn/dc = 0.136$, which was determined for cellulose in DMAc/LiCl (0.9% w/v) at 25 °C and 488 nm at BOKU, Vienna, Austria.

Table 3. HPSEC equipments used in publications I-V.

<table>
<thead>
<tr>
<th></th>
<th>I, II</th>
<th>III, IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPSEC</td>
<td>Alliance 2690 (Waters Corp., USA)</td>
<td>GPCmax (Viscotek Corp., USA)</td>
<td>Kontron pumps</td>
</tr>
<tr>
<td>Filtration</td>
<td>0.45μm</td>
<td>0.45μm (+0.2μm after columns)</td>
<td>0.45μm</td>
</tr>
<tr>
<td>Column</td>
<td>2 in series</td>
<td>2 with a guard column</td>
<td>4 in series</td>
</tr>
<tr>
<td></td>
<td>μHydrogel 2000+500+250 Å, 7.8x300mm (Waters)</td>
<td>OHpak SB-806M HQ, 8x300mm (Showa Denko)</td>
<td>Plgel 20μm Mixed-ALS</td>
</tr>
<tr>
<td>Eluent Rate</td>
<td>NaOH (0.05M), 60°C</td>
<td>NaNO3 (0.1M), 30°C</td>
<td>DMAc/LiCl (0.9%, w/v), RT</td>
</tr>
<tr>
<td>Eluent</td>
<td>0.5 ml/min</td>
<td>1 ml/min</td>
<td>1 ml/min</td>
</tr>
<tr>
<td>Inject. V</td>
<td>50 μl</td>
<td>50 μl</td>
<td>100 μl</td>
</tr>
<tr>
<td>Detectors</td>
<td>Fluorescence (M-474, Waters)</td>
<td>DALLS ($\lambda_e$=670 nm, 7 and 90 °C)+ Viscometric (270 dual, Viscotek)</td>
<td>RI (Shodex RI-71)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RI (VE 3580, Viscotek)</td>
<td>Fluorescence (Shimadzu, RS-535)</td>
</tr>
<tr>
<td>$dn/dc$</td>
<td>-</td>
<td>0.151 (Gomez et al., 1997a)</td>
<td>0.136</td>
</tr>
<tr>
<td>Software</td>
<td>Empower</td>
<td>OmniSEC 4.5</td>
<td>Astra 5</td>
</tr>
<tr>
<td>Calibr.</td>
<td>Series of β-glucan stds</td>
<td>LS/visc.</td>
<td>LS</td>
</tr>
<tr>
<td>Other</td>
<td>calcofluor dying after separation</td>
<td>pullulan std for detector calibr.</td>
<td>Fluorescence for c=o detection</td>
</tr>
</tbody>
</table>

Calculation of the degradation rate was based on the principle of first order kinetics, since every chain cleavage produces a reducing end i.e. one additional fragment (Hjerde et al., 1996). The random scission, i.e. chain cleavage without any preferential reaction sites, should obey the following equation:

$$\frac{1}{Xw,t} - \frac{1}{Xw,0} = \frac{k_d t}{2}$$  \quad \text{Equation 1}

where $x$ is the weight average degree of polymerisation of the chain at time $0$ or $t$, and $k_d$ is the rate constant for bond cleavage. As $x$ is proportional to the molecular weight, the equation 1 indicates that the reciprocal of the molecular mass should increase linearly with time, and the weight average molar mass ($M_w$) can be used as follows:
Materials and methods

\[
\frac{1}{M_{w,t}} - \frac{1}{M_{w,0}} = \frac{k_d t}{2}
\]

Equation 2

where \(M_{w,t}\) is the \(M_w\) of the treated \(\beta\)-glucan and \(M_{w,0}\) the \(M_w\) of the untreated \(\beta\)-glucan, \(k\) is the degradation constant and \(t\) is time. In the dilute regions \([\eta] = \frac{\eta_{sp}}{c}\), and in semi-dilute regions \([\eta] = 2\frac{\eta_{sp}}{c}\) (Doublier and Wood, 1995; Lazaridou et al., 2003), where \(\eta_{sp}\) is the specific viscosity (\(\eta_{sample}/\eta_{solvent}\)). The sample solutions with \(c[\eta]< 2.7\) (See Table 1: \(c^{[\eta]} = 2.7\)) were used for the degradation rate determination by the following equation (Equation 3), which is an estimation of equation 2. In addition, only the exponential phase of viscosity loss was taken into account.

\[
\left(\frac{c}{\eta_{sp,t}}\right)^{1/\alpha} - \left(\frac{c}{\eta_{sp,0}}\right)^{1/\alpha} = K_D t
\]

Equation 3

\[
K_D = \frac{k_d}{2M_0 \cdot K^{1/\alpha}} = 2.3k_d
\]

where \(M_0\) is the molar mass of the monosaccharide of \(\beta\)-glucan, and \(\alpha\) and \(K\) are the Mark-Houwinks exponent and constant, respectively. The Mark Houwink - equation \([\eta] = K (M_w)^\alpha\) for oat \(\beta\)-glucan is \([\eta] = 7.3 \times 10^{-4}(M_w)^{0.72}\) (Wood et al., 2000), and thus \(K_D = 2.3k_d\).

4.4.2 Analysis of oxidation (III, V)

The formation of free radicals (III) in the presence of ascorbic acid, ferrous ions and in heating at 95°C 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 at a concentration of 40 mM. Ethanol was added to oat bran extracts (4%) to increase the sensitivity and selectivity of detection, but in the case of OBG no additional ethanol other than that added in the solubilisation step, was needed. The sample solutions were kept at 21°C or in 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 (parameter in III). All spectra, consisting of four scans, were recorded at 21°C. 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 (2,2,6,6-Tetramethylpiperidine 1-oxyl) solution (2 μM). The TEMPO (stable radical reference, 99%, Sigma Aldrich) was used as a reference sample. All samples were analysed in triplicate.
The hydrogen peroxide content (III) of the heat-treated (95°C and 120°C) OBG and NBG without ferrous ion addition was analysed by a ferrous oxidation xylenol orange method (FOX) at given time intervals. The acidic FOX reagent (III) containing an excess amount of reduced iron (Fe²⁺) was prepared daily by mixing aqueous xylenol orange and d-sorbitol (0.137mM and 0.1 M, respectively) with a stock solution of FeSO₄, (NH₄)₂SO₄ and H₂SO₄ (25mM, 25mM and 2.5 M). The FOX reagent was combined with sample in ratio of 1:10 and incubated for 30 min at 21°C. The absorbance (A₁) of the xylenol orange-oxidized iron (Fe³⁺)-complex was measured at 560 nm (Perkin Elmer, Waltham, USA). The content of hydrogen peroxide was calculated against an external standard curve (2-100μg/l of H₂O₂) and the effect of the matrix was eliminated by subtracting the absorbance of acidified matrix. Since FOX-analysis also can measure other peroxides than H₂O₂ and iron complexes, catalase was added after the treatments at a concentration of 6000U/l to investigate whether the measured -equivalents were actually hydrogen peroxide and no other oxidizing compounds.

Oxygen consumption (III) following ascorbic acid addition at room temperature, by heat-treatments at 95°C and 120°C and following ferrous ion addition was estimated by measuring the amount of dissolved oxygen (mg/ml) of NBG and OBG using 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 21°C. The samples were treated (heating, ascorbic acid reaction) in closed vials (15 ml) with sample amount of 11 ml. Before the measurements, the samples were carefully cooled to 21°C and sealed. After the measuring, vials without headspace were resealed with parafilm.

Carbonyl group (c=O) formation (V) along the β-glucan chain was determined by labelling the existing carbonyl groups and analysing them after HPSEC-fractionation by a fluorescence detection (290 nm excitation, 340nm emission). The labelling compound CCOA (carbazole-9-carboxylic acid [2-(2-aminooxyethoxy)ethoxy]amide) was added to the treated OBG solutions, and treated+purified NBG solutions. The dried samples were dissolved in water (10mg/ml OBG, 1 mg/ml NBG) by shaking them overnight. A CCOA-stock solution (zinc acetate buffer at pH 4) and the sample solutions were combined to result in a CCOA concentration of 1.25 mg/ml. The solutions were agitated in a water bath for 168 h at 40 °C. Thereafter, unreacted label was removed by precipitation of the β-glucan with ethanol (60%) and washing thoroughly with 99% ethanol. Immediately after washing the sample, the β-glucan was dissolved in 1 ml DMAc/LiCl (0.9%, w/V) by shaking overnight at room temperature. 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. The total carbonyl content was calculated against six cellulose standards with known carbonyl group content labelled in the same series, which is presented as c(carbonyl)total. The content of carbonyl groups (μmol/g) formed along the chain was determined by estimating
the quantity of reducing end groups (μmol/g) as a reciprocal of the number
average molar mass ($M_n$) as follows:

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

### 4.4.3 Illustration of aggregates and assemblies of β-glucan

The role of aggregation (previously unpublished results) was examined with
dynamic light scattering (DLS) measurements using a BIC (Brookhaven
Instruments Corporation, Holtsville, NY, USA) light scattering setup composed of
a BI-200SM goniometer, a BIC-TurboCorr digital pseudo-cross-correlator, and a
BI-CrossCorr detector, including two BIC-DS1 detectors; pseudo-cross-
correlation functions of the scattered light intensity were collected with the self-
beating method A Sapphire 488-100 CDRH laser (Coherent GmbH) operating at
$\lambda_o = 488$ nm and with the power adjusted in the range from 10 to 50 mW was
used as the light source. The measurements were performed at a scattering
angle $\theta=90^\circ$ and 20°C. The treated NBG solutions were diluted 1:1 with 1 M
NaOH and equilibrated overnight. The alkaline solutions were filtered directly
into the cylindrical sample cuvette (glass, diameter 10 mm) using hydrophilic
Millex-HV filter units with a pore size of 0.45 μm, and measured after 20-30 min
equilibrating time. The typical duration of the measurements was 15 min. Size
distributions were obtained using a multi exponential fit provided by an inverse
Laplace transform program CONTIN. Mean peak values of the size distributions
were used to estimate the apparent hydrodynamic radius, $R_{h,\text{app}}$. The true
hydrodynamic radius at $\theta = 0$ and $c = 0$ was not estimated. Further
methodological aspects of light scattering methods are presented in Chu (1991)
and Brown (1993).

Illustration of β-glucan assemblies (II, IV) in stored NBG was performed by
confocal scanning laser microscopy (CSLM) using calcofluor dying. The fresh,
homogenised (HPVH, 60 MPa, 2 min) samples stored for 2 weeks (+6 °C) were
each mixed with 20 μl/l calcofluor (100 mM sodium carbonate buffer, pH 10) at a
ratio 1:1 (v/v). The mixtures were pipetted onto microscope glass slides and
imaged with confocal scanning laser microscopy (Leica TCS SP2 AOBS, Germany)
with an excitation wavelength of 350 nm and an emission wavelength of 420 nm.
Analyses were performed in triplicate.
5 RESULTS

The changes in viscosity properties of solutions of highly purified (OBG) and native β-glucan with co-extracted compounds (NBG) were identified after treating the solutions with common food additives and processes. These treatments caused considerable changes in the solution properties, which were further investigated by the molecular parameters ($M_w$, $R_g$, $R_h$, $\alpha$) of β-glucan. Enzymatic and acid hydrolysis were excluded by the study design and the contribution of oxidative reactions to the chain cleavage was examined by using various methods. The carbonyl group determination method was based on labelling of the carbonyl groups and enabled the simultaneous determination of carbonyl group formation and molar mass decrease. This was the first time this method was used for a water-soluble polysaccharide.

5.1 CHEMICAL COMPOSITION OF THE SOLUTIONS

The dry weight of NBG consisted of approximately 40% β-glucan, 60% total carbohydrates, and 15% proteins (Table 4). Trace amounts of lipids and transition metals were detected, and the relatively high content of phosphorus was associated with phytic acid or phytate (myoinositolhexaphosphate). When all the phosphorus was assumed to originate from phytic acid, the content of phytic acid was approximately 5% of the dry weight of the fresh NBG, since phosphorus accounts for 32% of the molar mass of the myoinositol molecule. When the β-glucan was isolated from NBG by ethanol precipitation (NBGp), the β-glucan content increased to 80–90% and protein content decreased from 15% to 5%. The molar mass of β-glucan in untreated NBG was approximately 1 500 000 g/mol and in the commercial highly purified β-glucan (OBG) 260 000 g/mol (Table 5). The values of the term $c[\eta]$ were 0.9–1.8 for NBG with 1–2 mg/ml of β-glucan and 2.3–2.6 for OBG with 7–10 mg/ml of β-glucan, and thus the studied solutions were in the semi-dilute or region $c^* < c < c^{**}$ (see Table 1).

No β-glucanase activity was detected from the NBG solutions (II), which was used to examine non-enzymatic alterations during processing. The milling process of oat bran concentrates generally includes heating steps to inactivate the enzymes, and the oat bran concentrate used for NBG includes an additional hot ethanol treatment. Samples stored for four weeks were also found to be enzyme-free, and the viscosity decrease of NBG during 1, 2, 3 and 4 weeks at +6 °C (II, Fig. 1a) was associated with non-enzymatic degradation (II, Fig. 1b) or aggregation-induced compacting of the molecules (II, Fig. 2).
Table 4. Total carbohydrates, proteins and fatty acids and selected minerals in a solution of the native β-glucan extracted from oat bran (NBG), in solution of commercially highly purified β-glucan (OBG) and in NBG with a ethanol precipitation step (NBGp).

<table>
<thead>
<tr>
<th></th>
<th>NBG*</th>
<th>NBGp</th>
<th>OBG</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-glucan (%, w/dw)**</td>
<td>40±4</td>
<td>87±3</td>
<td>99</td>
</tr>
<tr>
<td>Carbohydrates (%, w/dw)***</td>
<td>58±2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (% of total)</td>
<td>86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galactose (% of total)</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabinose (% of total)</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xylose(% of total)</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteins (%, w/dw)</td>
<td>15±1</td>
<td>4±1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Fatty acids (%, w/dw)</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:0 (% of total)</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:0 (% of total)</td>
<td>71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minerals (mg/100g dw)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>2.5</td>
<td>4.1</td>
<td>13.2</td>
</tr>
<tr>
<td>Cu</td>
<td>2.4</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Zn</td>
<td>4.5</td>
<td>2.2</td>
<td>3.1</td>
</tr>
<tr>
<td>P</td>
<td>1900</td>
<td>900</td>
<td>390</td>
</tr>
</tbody>
</table>

dw=dry weight, *1-2 mg/ml β-glucan, ** enzymat. analysis, ***HPLC analysis after hydrolysis

5.2 ASCORBIC ACID INDUCED DEGRADATION OF β-GLUCAN (I,II,III,V)

The viscosity [$\eta_{app}(10s^{-1})$] decrease due to carboxylic acids that commonly occur in foods (malic, citric and ascorbic acid) was examined at beverage-related concentrations and pH-conditions (pH 4.1-4.7) during storage. Ascorbic acid (2mM) caused a considerable decrease in the viscosity of NBG unlike the other carboxylic acids at a similar concentration (Fig. 5A, II, Fig. 3). The viscosity of NBG decreased to 1-5 mPas after a day of storage at the latest, irrespective of the ascorbic acid concentration (1-50 mM), reaction temperature (6-120°C) or β-glucan concentration in the NBG-solution (1-2 and 4-5 mg/ml, $\eta_{app}(10s^{-1})=80$ and 800mPa·s, Fig 5B). The flow behaviour was shear thinning in the untreated NBG-solutions, but turned to Newtonian in the presence of ascorbic acid (Fig. 5A). This indicates a chain cleavage or conformational changes in the treated β-glucan, due to which the molecules were no longer able to entangle in the solution. The rate of the viscosity decrease of NBG was more dependent on the reaction temperature (21°C or 6°C) than the ascorbic acid concentration (2, 10 or 50 mM (Fig. 5C). The ascorbic acid (10mM and 50mM) induced viscosity loss was also considerable in the solution of purified β-glucan (OBG), although the degradation rate was 5-10 times slower than NBG (Figs 5B and 5C), for which the degradation rate $k_d$ was in order of $10^{-5}$ min$^{-1}$. The experiments of OBG were performed with added ferrous ions to examine the role of these oxidation catalysts. The presence of ferrous ions significantly enhanced the viscosity decrease in cold storage (6°C), but not at 21°C, 95°C and 120°C (III, Table 3). Despite of the labile nature of
Results

Ascorbic acid, it altered the degradation of β-glucan also at these pasteurisation and sterilisation temperatures (95°C and 120°C; III, Table 3).

Figure 5. A) The flow curves of untreated and ascorbic acid-treated (2 mM) NBG after cold storage, B) Ascorbic acid-induced viscosity loss of NBGx and OBGc [c = c(β-glucan) (mg/ml)] at 21 °C (RT), or at 6 °C (cold). The NBG4 is shown on the right-hand y-axis and the error bars are for three replicants. C) Rate of β-glucan degradation calculated from the data of Fig. 1B by Eq. 3, where the slopes are the degradation rates k_d (min⁻¹). In Figures B and C the solid lines represent 10 mM, the dotted lines 50 mM and the dashed lines 2 mM additions of ascorbic acid. The pH of the samples was 4.0±0.5.
Results

5.2.1 Molecular changes of β-glucan in ascorbic acid treatments (III, V)

A considerable decrease in the molar mass of β-glucan was obtained when NBG was treated for 2-5 hours (21°C) with 1 mM ascorbic acid, as the molar mass decreased from 1400 000 g/mol to 380 000 g/mol (Table 5). The decrease was further enhanced by ferrous ion addition. After a reaction time of several days, the molar masses of β-glucan in NBG were low or no longer detectable (Table 5). In OBG, the molar mass resulted in a slight decrease (from 280 000 g/mol to 260 000 g/mol) after a treatment (1 mM ascorbic acid at 21 °C) time of hours, but was significant after days of treatment (Table 5). These molar mass results are indicative, and demonstrate the degradation. Accurate Mw values at certain time points were not attained in this study, since the degradation reactions continued in the analysis, which had a duration of 1 h for each sample.

The conformation of β-glucan, determined by the Mark-Houwink exponent (α), remained as a random coil in the ascorbic acid treatments of NBG and OBG at 6°C and 21°C. In the HPSEC-system using an organic solvent and MALLS-detection (V), the conformation was determined as the slope in the plot of the radius of gyration (Rg=R RMS = radius of root mean square) and Mw, indicating the apparent density of the molecule. The conformation of β-glucan in ascorbic acid treated (21°C) NBG was random coil (slope 0.6-0.7), but the appearance of compact high molar mass species (Mw>10^5.5 g/mol) was observed as a bending of the conformation plot similarly as shown for heat-treated samples (V, Fig. 2a).

Table 5. Selected weight average molar masses (Mw, 10^3 g/mol) obtained by the different HPSEC-methods of publications I-V after the ascorbic acid, thermal and high pressure homogenisation treatments of OBG and NBG, and in the storage of NBG.

<table>
<thead>
<tr>
<th>Mw</th>
<th>NBG</th>
<th>OBG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unchanged</td>
<td>1500</td>
<td>280</td>
</tr>
<tr>
<td>AA 1-2mM</td>
<td>50^ a</td>
<td>380^ b</td>
</tr>
<tr>
<td>AA 2mM+Fef</td>
<td>&lt; 30^ a</td>
<td>210^ b</td>
</tr>
<tr>
<td>AA 10 mM</td>
<td>50^ a</td>
<td>380^ b</td>
</tr>
<tr>
<td>95°C 30 min</td>
<td>1200</td>
<td>270</td>
</tr>
<tr>
<td>120°C 30 min</td>
<td>710*</td>
<td>230</td>
</tr>
<tr>
<td>120°C 30 min+Fef</td>
<td>390</td>
<td>180</td>
</tr>
<tr>
<td>120°C 15 min</td>
<td>630*</td>
<td>250</td>
</tr>
<tr>
<td>100 MPa 5 min</td>
<td>150</td>
<td>110</td>
</tr>
<tr>
<td>30 MPa 5 min</td>
<td>150</td>
<td>140</td>
</tr>
<tr>
<td>2 wks stored</td>
<td>1200</td>
<td>1300</td>
</tr>
<tr>
<td>5 wks stored</td>
<td>1300</td>
<td>1300</td>
</tr>
</tbody>
</table>

^ a The exact reaction time is unknown (days), ^ b Reaction time 2-5 h, η_app, η=150 mPa·s, ^ c Reaction time of 4 h, η_app, η=600 mPa·s, ^ d Reaction time 4 days, fc(FeSO4)=0.05-0.1 mM, * Aggregation observed
**5.2.2 Oxidative reactions in solutions containing ascorbic acid (I,II,IV)**

The oxidative reactions examined were Fenton type reactions, where hydrogen peroxide, ferrous ions, dissolved oxygen and hydroxyl radicals play a key role (See Reactions 1-4). The ascorbic acid induced viscosity loss of NBG was slowed down by the competitive hydroxyl radical scavengers glucose (0.5 M) and mannitol (0.5 M, Fig. 6; II table 1). The effect of the hydrogen peroxide decomposer, catalase, was similar to that glucose and mannitol, as it slowed down the viscosity loss of NBG (Fig. 6; II table 1). In contrast, the addition of the metal chelator phytic acid, accelerated the ascorbic acid induced viscosity loss of NBG. In the solutions of purified β-glucan, the inhibition of the viscosity loss by glucose (1 M) was almost total in a 16 hours experiment (I, Fig. 1).

The free radical formation was boosted in NBG (Fig. 7A) and in OBG+Fe²⁺ (Fig. 7B) by the addition of ascorbic acid (1 mM, 95 °C). Consistently, the viscosity of NBG and OBG respectively decreased by 90% and 50% more with ascorbic acid than without, despite the occurrence of ferrous ions, after treatment at 95 °C (30 min). Furthermore, the molar mass of β-glucan in NBG and OBG was 170 000 g/mol and 200 000 g/mol, respectively, after 2-5 hours of treatment at 95°C (30 min) in presence of 1 mM ascorbic acid, compared to the 270 000 g/mol and 1200 000 g/mol without ascorbic acid.

The formed free radicals were most likely hydroxyl radicals, since the use of ethanol in the study solution. Ethanol rapidly donates the unpaired electron of the free radicals, but the reaction requires an oxidation potential over 1200 mV. Hydroxyl radicals are practically the only radical species with sufficient oxidation potential (E(·OH) =2320 mV), and are thus selected to the spin trapping. The addition of ascorbic acid (1,2,5,10 and 50 mM) also caused the consumption of
dissolved oxygen, when determined at 21°C from NBG. All the treatments consumed 46-57 μM of oxygen in 15 minutes and 67-82 μM in 3 hours.

**Figure 7.** Radical formation at 95°C in presence of 0.05 mM FeSO₄ and/or 1 mM of ascorbic acid (AA1) in A) NBG and B) OBG. The error bars are the standard deviation of three replicates and are not visible in control samples.

### 5.2.3 Carbonyl group formation in ascorbic acid treatments (V)

The formation of the oxidized hydroxyl groups was significant in the ascorbic acid treated OBG (4 days reaction time) as the carbonyl group content was 15-20 μmol/g (V, Table 2). The molar mass simultaneously decreased from 260 000 g/mol to 62 000 g/mol and 67 000 g/mol by ascorbic acid concentrations of 10 mM and 50 mM respectively (Table 5). In NBG, the similar treatments (4 hours reaction time) resulted in approximately 5-7 μmol/g of carbonyl groups along the β-glucan chain with molar mass decrease from 1600 000 g/mol to 550 000 g/mol and 750 000 g/mol (Table 5; V, Table 2). The carbonyl groups mostly occurred in the low molar mass species, but interestingly, in the case of OBG, the carbonyl groups also formed along the chain of the high molar mass species in considerable amounts (Fig. 8).
Results

Figure 8. The carbonyl group (dashed line, $d_{\text{C=O}}$ = degree of carbonyl group substitution) and molar mass distribution (solid line) of β-glucan in DMAc/LiCl after the solution of highly purified β-glucan (OBG) was treated with ascorbic acid (10 mM, AA10) for 4 days and the solution of native β-glucan for 4 hours.

5.3 THERMAL DEGRADATION OF β-GLUCAN (III)

Thermal treatments of the β-glucan solutions decreased the viscosity of the solutions and the molar mass of β-glucan. The rate of the degradation was moderate or negligible at 95°C, but significant at 120°C, and ferrous ions had a significant effect on the rate (Fig. 9). In most cases, the molar mass loss was in linear relation with the treatment time (Fig. 9). At 120°C (NBG, OBG+Fe$^{2+}$), where the degradation was most intensive, the slope i.e degradation rate constant $k_d$, resulted in $k_d=10^{-5}$ min$^{-1}$, and thus corresponded to the rate of ascorbic acid induced degradation (Figs. 5B and 9).

Figure 9. Rate of thermal degradation of β-glucan with and without Fe$^{2+}$ ions (Fe), when calculated by Eq. 2 as the change in the molar mass of β-glucan in NBG (solid lines) and OBG (dashed lines) during the treatments. No linear relation was found in the degradation of β-glucan for NBG+ Fe$^{2+}$ at 120 °C (solid red lines). The points are averages of two replicates.
Results

Compared with the viscosity and molar mass losses in NBG, the changes in OBG were small (Fig. 9; Table 2, OBCet=NBG and MVO=OBG). For example, the treatment of 120°C for 30 minutes decreased the molar mass of β-glucan in OBG from 270 000 g/mol to ≈ 230 000 g/mol and in NBG from 1400 000 g/mol to ≈600 000 g/mol (Table 5). The molar mass decreased simultaneously with the viscosity in both NBG and OBG solutions when the viscosities were monitored at t=5 min at 120°C and t=30 min at 95°C (Fig. 1), indicating that the viscosity loss was due to the chain cleavage and not conformational changes. However, when the exposure time at 120°C was extended up to 30 minutes, the values of $M_w$ of NBG were ambiguous due to the detected double peaks (Fig. 2). In the presence of ferrous ions or in the organic solvent, only one population was obtained throughout the study (Fig. 2; V, Fig. 1a), and thus the degradation rate of NBG at 120°C was calculated from the $M_w$-values obtained by the organic solvent (Table 5).

When the ferrous ions concentration was relatively high (1 mM), the treatment of OBG at 120°C for 30 minutes resulted in an $M_w$ of 90 000 g/mol. Furthermore, a second population with high molar mass species in order of $M_w > 10^{6.5}$ g/mol was interestingly obtained (V, Fig. 2b). Similarly to the ascorbic acid treated samples, the conformation of high molar mass species or clusters in the molar mass range of $≈10^{5.5}$ g/mol of heat-treated β-glucan in OBG was compact as seen by the bending curve of the conformational plot (V, Fig. 2a).

5.3.1 Oxidative reactions in the thermal treatments (III)

Thermal treatment (95 °C) initiated free radical formation in both OBG and NBG solutions (Fig. 10). At room temperature, no radicals were detected up to a reaction time of 1.5 hours. The free radical formation had a higher rate in NBG compared to OBG and the rate increased following the addition of ferrous ions, consistently with the viscosity and molar mass decrease (Fig. 3). Hydrogen peroxide was also formed during the heat-treatments, especially in the NBG solution and at 120°C (III, Fig. 4, OBCet=NBG, MVO=OBG). Oxygen was consumed at levels of 90 μM during the 30 minutes treatment at 120°C in both solutions, and 30 μM at 95°C, and increased further in the presence of added ferrous ions. In addition to free radical formation, hydrogen peroxide formation and oxygen consumption, the contribution of oxidation reactions to the thermal degradation was investigated by using of antioxidants. Sulphite had a protective effect on the viscosity loss of NBG at 120°C, but the inhibition effect was most effective when sulphite was used with ascorbic acid in the solutions to be heated (III, Table 3, MVO=OBG and OBCet=NBG).
Results

Figure 10. The ESR-spectra of POBN spin adducts formed in NBG and OBG samples with and without FeSO₄ (0.05 mM) after 15 minutes at 95°C.

5.3.2 Carbonyl group formation during heating (V)

Heating for 30 min at 120°C resulted in a significant carbonyl group content (≈10 μmol/g) along the β-glucan chain in NBG (V, Table 2). In OBG+Fe²⁺, the content of carbonyl groups was 15 μmol/g with an exposure time of 15 min, but decreased to ≈5 μmol/g by extending the exposure time to 30 min (V, Table 2). This is also seen in Figure 11, as the degree of carbonyl group substitution (dₛ_C=O) was at a higher level throughout the molar mass range after 30 minutes than after 15 minutes of treatment at 120 °C. At 95 °C, no carbonyl group formation was detected in NBG or in OBG without ferrous ion addition (V, Table 2).

Figure 11. The carbonyl group (dashed lines, dₛ_C=O = degree of carbonyl group substitution) and molar mass distributions (solid lines) of β-glucan in DMAc/LiCl after heating the water solution OBG (120°C, 15-30 min) with and without 1 mM FeSO₄. The gray carbonyl group substitution line of the untreated sample is invisible, since its similarity with the black dashed line.
Results

The contribution of the co-extracted compounds (present in NBG) was studied by purifying the β-glucan from the extract by ethanol precipitation and dissolving it in pure water before heating at 120°C for 15 minutes (NBGp). In the sample without the purification step, the carbonyl groups formed along the chain at higher concentration, were distributed over the whole range of molar mass species, and the molar mass decreased more significantly than in the purified sample (Fig. 12).

![Figure 12](image)

**Figure 12.** The carbonyl group (dashed lines, $d_{sC=O}$ = degree of carbonyl group substitution) and molar mass distributions (solid lines) of β-glucan in DMAc/LiCl after heating NBG (120°C, 15min) with and without the purification before the treatment.

5.4 DEGRADATION OF β–GLUCAN IN HOMOGENISATION (IV,V)

The viscosity of NBG and molar mass of β-glucan decreased in proportion to the homogenisation pressure ($P$) depending on the type of equipment, exposure time and the original viscosity of the fluid (Fig. 13; IV, Fig. 4). Microfluidizer (MF) treatment caused more intensive degradation than traditional high pressure valve homogenisation (HPVH, Fig. 13). The molar mass of β-glucan decreased in the relation of $P \sim M_w^{-0.5}$ as a result of the HPVH-treatment (2 min) and $P \sim M_w^{-0.6}$ following the MF-treatments (IV, Fig. 4). In addition, a linear relationship was found with the molar mass decrease and exposure time the the MF-treatments at 30 MPa and 100 MPa (Fig. 14), with degradation rates $k_d$ in order of $10^{-3}\text{ min}^{-1}$ and $10^{-4}\text{ min}^{-1}$, respectively.
Results

Figure 13. The rate of β-glucan degradation as a function of homogenisation pressure when calculated by Eq. 3 as a change of viscosity (solid lines) and by Eq. 2 as a change of molar mass (dashed lines) in NBG with 1.5mg/ml of β-glucan ($\eta_\text{app},0 \approx 100 \text{mPas}$).

The treatments with the maximum pressures of HPVH (60 MPa=600 bar) and MF (100 MPa=1000 bar) resulted in weight-average molar masses of 300 000 g/mol when treated for up to 2 minutes. The MF treatment by 100 MPa with exposure times of 5 and 10 minutes resulted in $M_w \approx 150 000$ g/mol and narrowed the molar mass distribution of β-glucan in NBG (Fig. 14). Corresponding molar masses were found after treating OBG, which had a significantly lower original molar mass of β-glucan but a similar solution viscosity to NBG, at 100 MPa for 5 minutes (Table 5).

Figure 14. The molar mass distributions of microfluidizer (MF)-treated β-glucan in NBG ($\eta_\text{app},0=100 \text{mPas}$). The effect of increasing chamber pressure (30 MPa, 50 MPa and 100 MPa) in one-cycle treatment (exposure time 10 s) is shown by the bolded lines, and effect of increasing exposure time (5 minutes and 10 minutes) at pressure 100 MPa=1000 bar is shown by the red lines.
In addition, homogenisation interestingly made the assemblies more uniform and inhibited the formation of large aggregates (IV, Fig. 7), which were formed in the storage (6°C) of NBG (II, Fig. 2). This phenomenon illustrated by calcofluor dyeing and confocal laser scanning microscopy, indicates the enhanced dispersion of β-glucan after homogenisation.

**Carbonyl group formation during homogenisation (V)**

The contribution of oxidation to the homogenisation-induced degradation was studied as the carbonyl group formation along the β-glucan chain in the treatment of 5 minutes with the highest and lowest MF pressures (30 and 100 MPa). The carbonyl group content increased to 5-10 μmol/g along the chain of β-glucan following all the treatments, the increase being more significant on OBG. The carbonyl groups distributed through the entire molar mass range (V, Fig. 4). Similarly to the heat-treated and ascorbic acid treated OBG samples, the slope of the conformation plot (log $R_{RMS}$/log $M_w$) was bent similarly to the ascorbic acid and heat-treated samples, indicating a compact conformation of the high molar mass species. Unlike after the other treatments, the high molar mass species of homogenised NBG also appeared to have a compact conformation.

**5.5 AGGREGATION OF β-GLUCAN**

The possible aggregation of β-glucan at the time of analysis may cause errors to in the conclusions regarding the degradation, since the aggregation of even a small proportion of the molecules in solution easily leads to over-estimation of molar mass values determined by light scattering techniques. Thus, the role of aggregation was carefully considered throughout the study. The light scattering signals (LALLS and RALLS, and MALLS -detectors) and molar mass populations of untreated OBG and NBG resulted in one population without significant or systematic splits or shoulder in the peaks (See Figs 8, 11, 12, 14 and V, Figs 1, 3 and 4), and did not, therefore expose the aggregation. The negligible or low degree of aggregation was suggested by the similar molar mass results obtained by aqueous and organic eluent, due to the aggregate dissociating effect of the organic solvent used, DMAc (Table 5).

The value of shape parameter ($\rho=R_g/R_h$) of β-glucan (III, IV) was in the range of 1.5–1.7 in both the OBG and NBG solutions after homogenisation and most heat-treatments, also indicating a low degree of aggregation. In addition to HPSEC-LS detection, the role of aggregation was also examined by dynamic light scattering (DLS) in selected samples of NBG (previously unpublished results). Samples were analysed in 0.5M NaOH, which disintegrates the aggregates. The β-glucan in homogenised (IV, microfluidizer, 1 pass, 30, 60, 100MPa) and heat-treated (III, 95°C for 30 min and 120°C for 5 min) NBG showed up in two populations with low and high values of hydrodynamic radius ($R_h$) values. Of these populations,
the one with low value of $R_h$, assumingly the population with a low degree of aggregation, agreed with the values of $R_h$ obtained by HPSEC (Fig. 15).

Based on the results and the present knowledge, the molar mass values obtained with the HPSEC-techniques in the present study seems to represent the molar mass of a single or insignificantly aggregated β-glucan and are reliable for the estimations of the degradation intensity.

**Figure 15.** The size of β-glucan in the NBG solution after heat treatment (120 °C, red line) or microfluidizer high-pressure homogenisation (30 MPa and 100 MPa, black and blue lines, respectively). The hydrodynamic radius ($R_h$) obtained by dynamic light scattering (DLS) was determined in 0.5M NaOH, and HPSEC analysis was performed with the aqueous eluent
6 DISCUSSION

In aqueous solutions, the β-glucan molecule was cleaved by ascorbic acid treatment, heating and high pressure homogenisation. The Investigation of oxidation as a relevant degradation mechanism of β-glucan was driven by knowledge of the pro-oxidant nature of ascorbic acid. Several oxidation parameters such as free radical and hydrogen peroxide formation, oxygen consumption and the accelerating effect of ferrous ions indicated the contribution of oxidative reactions to the degradation. The carbonyl group formation along the β-glucan chain with consistent chain cleavage demonstrated that oxidation did have a key role in the process-related degradation of β-glucan.

6.1 ASCORBIC ACID INDUCED OXIDATION OF β-GLUCAN

Already at juice-related concentrations of ascorbic acid the viscosity of the semi-dilute and concentrated β-glucan solutions effectively decreased (Fig. 5A). The viscosity of an oat β-glucan extract (NBG) resulted in water-like viscosities in time-scale of hours at room temperature in the presence of 1-50 mM of ascorbic acid. Similar trends of viscosity loss were previously demonstrated for aqueous solutions of other water-soluble glucans such as cassava starch (Valles-Pamies et al., 1997), pullulan (Cercenzi et al., 1997), xyloglucan (Fry, 1998), chitosan (Zoldners et al., 2005) and CMC (Crescenzi et al., 1997; Fry et al., 1998).

In general, a linear relationship between the reciprocal of the estimated molar mass and time, was found in all studied conditions (Fig. 5B). This agrees with the kinetic trend of both acid hydrolysis and oxidative cleavage of the single-stranded polysaccharides and indicates a random scission mechanism (Hjerde et al., 1994; Rickards et al., 1967; Wang et al., 2000). The degradation rate was more dependent on the reaction temperature and solution properties (co-extracted compounds) than the ascorbic acid concentration (Fig. 5B). The β-glucan degradation rate in NBG in the presence of 10 mM of ascorbic acid was in order of $10^{-5}$ min$^{-1}$, and 5-10 times lower in the solution of purified β-glucan (OBG). Crescenzi et al. (1997) recorded degradation rates of order of $10^{-4}$ min$^{-1}$ for purified pullulan and CMC in presence of ascorbate (+CuSO$_4$). This suggests a relatively stable nature of oat β-glucan against ascorbic acid treatment, although an immediate and considerable effect was evident. Usually, the degradative effect of ascorbic acid has been shown at room temperature, but here ascorbic acid was also observed to accelerate the degradation of β-glucan at pasteurization and sterilization temperatures (95 and 120°C; III, Table 3), despite of the labile nature of ascorbic acid.
Ascorbic acid is known to cause oxidative cleavage of macromolecules, since it can induce the hydrogen peroxide mediated hydroxyl radical production, which can further attack the macromolecule (Scheme 6; Fry, 1998; Valko et al., 2005). The ascorbic acid addition significantly accelerated the formation of the hydroxyl radicals (Figs. 7A and 7B), demonstrating the pro-oxidativity of ascorbic acid. These results agree with the ascorbic acid effect obtained in beer (Andersen et al., 2000). In addition, the ascorbic acid-induced viscosity loss was slowed down by the addition of glucose (Fig. 6), which supports the hypothesis of cleavage of β-glucan resulting from hydroxyl radical attack induced in solutions containing ascorbic acid. Glucose can act as a competitive hydroxyl radical scavenger, since it reacts more readily with the short-living hydroxyl radicals than is expected for the β-glucan chain (Buxton et al., 1998). However, glucose and mannitol did not fully eliminate the ascorbic acid induced viscosity loss of NBG, which was the case in the pure β-glucan solution (I, Fig. 1), and has been reported for pure xyloglucan and dextran (Fry, 1998; Schweikert et al., 2000). This indicates the contribution of the co-extracted compounds of NBG to the degradation of β-glucan.

In addition to hydroxyl radical formation, dissolved oxygen was consumed by the addition of ascorbic acid in significant amounts (70-80 μM in 3 hours by 1-50 mM ascorbic acid in NBG). The oxygen consumption is related to the oxidation processes, because the dissolved oxygen is consumed in the peroxide formation in the radicalized molecule (Scheme 3), but also in the production of the free radicals by the reducing agents (Scheme 6). The reduction of the dissolved oxygen by ascorbic acid generally results in formation of hydrogen peroxide. The role of hydrogen peroxide was studied by catalase, which is an enzyme that can decompose hydrogen peroxide. Catalase significantly slowed down the ascorbic acid induced viscosity decrease (Fig. 6), which indicates that the Fenton chemistry (Reaction 1) contributed to the degradation of β-glucan in presence of ascorbic acid. The inhibition was only partial in NBG, similarly to the competitive hydroxyl radical scavengers. In the case of pure xyloglucan, the ascorbic acid-induced viscosity decrease was completely inhibited by similar catalase-treatment to that used in the present study similarly to the total inhibition effect of glucose (Fry, 1998). This highlights the complexity of the natural matrices and suggests that Fenton reaction only partially explains the ascorbic acid induced viscosity decrease of NBG.

In addition to the occurrence of the oxidative reactions in the β-glucan solutions, oxidation took place via the conversion of the functional groups of β-glucan to carbonyls in the ascorbic acid containing solutions (Fig. 8). The concentration of carbonyls after 4 days reaction time in OBG was ≈20 μmol/g, which is a significant amount, and corresponds to the concentration observed in electron-beam irradiated cellulose (Potthast et al., 2003). Ascorbate treatment (8 hours) of xyloglucan induced the formation of 15 oxidized groups/1000 glucose residues of the chain (Fry et al., 2001), corresponding to a carbonyl group concentration of 30 μmol/g, which agrees with the present study. The slightly higher content in
relation to the shorter reaction time may be explained, alongside the methodological differences, by the used hydrogen peroxide and copper sulphate. This is because hydrogen peroxide used with ascorbate boosted the viscosity decrease of a xyloglucan solution (Fry, 1998), and copper has been determined to be an 80 times more efficient catalyst in hydroxyl radical production than iron (Buettner and Jurkiewicz, 1996). Interestingly, ascorbic acid not only increased the carbonyl group content of OBG, but caused a sharp increase in the degree of substitution of the highest molar mass species (Fig. 8). Thus, the ascorbic acid treatments resulted in carbonyl group formation, which led to chain cleavage, but also in less degraded high molar mass species carrying carbonyl groups along the chain.

Scheme 6. Hypothesis of ascorbic acid induced carbonyl group formation and oxidative cleavage of β-glucan via metal-catalysed hydroxyl radical production (von Sonntag, 1980; Gratzl, 1987; Fry, 1998; Guo et al., 2002). The scheme presents the hydroxyl radical attack on C-1 in conditions which favour the fragmentation mechanism. This is one of the several possible reactions of the random attack of the hydroxyl radicals on β-glucan in the solutions.

The long duration of the oxidative reactions was indicative of the ascorbic acid induced cleavage of β-glucan. The degradation continued, as indicated by the molar mass results (Table 5), after the lag phase of the viscosity loss was reached (Fig. 5B). After a reaction time of ≈5 hours in NBG at room temperature, the molar mass of β-glucan was approximately 400 000 g/mol, while it was only 50 000 g/mol after 2–5 days of storage (Table 5). Similarly, in OBG, a reaction time of 5 hours resulted in an $M_w$ value of 250 000 g/mol, and after 4 days in 100 000 g/mol. This indicates that the hydroxyl radical production continued and the reagents were not consumed during the long reaction times. Hydrogen peroxide is an unstable compound, like ferrous ions and hydroxyl radicals. Ascorbic acid has been suggested to stabilise the hydrogen peroxide in water solution (Arts et al., 1997) and maintain the autoxidation cycle of iron (Buettner and Jurkiewicz, 1996), thus maintaining the reaction chains and oxidation. In
addition, as the oxidation progresses, superoxide (O₂⁻) is formed, which may further contribute to the oxidation reactions in the slightly acidic conditions by progressing to hydrogen peroxide (Scheme 6; Vreeburg and Fry, 2005).

The degradation rate of β-glucan followed the order of 2 mM < 50mM < 10 mM of added ascorbic acid in all the reaction temperatures and β-glucan solutions studied (Fig. 5B). Correspondingly with the molecular degradation, the degree of carbonyl group formation increased in the same order of added ascorbic acid (V, Table 2). No clear cross-over effect, where the pro-oxidativity of ascorbic acid turns to antioxidativity (Rees and Slater, 1987), was found in the present study, but the lower oxidation intensity of 50 mM than 10 mM of ascorbic acid may be explained by the increased rate of ascorbyl radical (A•) formation at the higher concentration of ascorbic acid. The antioxidative effect of ascorbate is due to the relatively stable ascorbyl radicals, which are able to cleave the oxidative radicals. The pro-oxidativity of ascorbic acid is associated with “low” concentrations of ascorbate, when hydroxyl radicals are produced, but the level of ascorbyl radicals is still negligible (Buettner and Jurkiewicz, 1996). At the concentration of 50 mM, ascorbate possibly produced ascorbyl radicals in amounts able to scavenge a proportion of the formed hydroxyl radicals and thus slowed down the degree of hydroxyl radical attack on β-glucan. In addition, ascorbic acid as a small molar mass compound may just simply act as the competitive scavenger similar to the small molar mass carbohydrates like mannitol and glucose.

6.2 THERMAL OXIDATION OF β-GLUCAN

The heating of the β-glucan solutions at 120°C caused a considerable viscosity and molar mass losses especially in NBG, and the effect was enhanced by the ferrous ions (Table, 5; III, Table 2, OBCet = NBG and MVO = OBG). The rate of thermal degradation of β-glucan was determined by the decrease in Mₚ, and the typical linear relationship between the reciprocal of the molar mass and time (Lai et al., 2000) was observed for most of the treatments (Fig. 9). At 120 °C (OBG +Fe²⁺, NBG), the rate of thermal degradation in order of 10⁻⁵ min⁻¹ corresponded with that reported for κ-carragenan at 75–85 °C, but was two orders of magnitude lower than reported for guar gum (Lai et al., 2000). Correspondingly, the viscosity of NBG decreased by only one order of magnitude at 120 °C (V, Fig. 5), while the viscosity with similar starting viscosities of guar gum solutions decreased by approximately 2 orders of magnitude in 60 minutes at 100 °C, 110 °C and 120 °C (Kök et al., 1999). These results indicate that oat β-glucan was also relatively stable against thermal degradation, as previously suggested by Autio et al. (1989). However, they reported a D-value (the time point, at which 90% of the viscosity is lost) of 145 hours for β-glucan at 100°C, while the equivalent value calculated from the data of Figure 5a (V) was found to be 5-10 hours for both OBG and NBG solutions at 95°C.
At 120°C, especially in the NBG solution with ferrous ions, the degradation of β-glucan had an initial exponential phase with high degradation rate, which was followed by a lag phase (Fig. 9, red solid line). This may indicate rapid degradation reactions and the consumption of reagents during heating in this unpurified solution. In addition, a specific limiting molar mass of the polysaccharide, under which the molecule is less sensitive to thermal degradation in the study conditions, can explain the degradation trend of NBG at 120°C. Bradley et al. (1989) found a higher rate of thermal degradation for guar gum in solution concentrations above the critical concentration c* than under c*, which was explained by the higher sensitivity of the entangled chains for the thermal degradation. The molar mass and viscosity of NBG rapidly decreased and the viscosity turned to Newtonian following the treatment at 120°C, indicating the approach of c<c* and disappearance of the entangled molecules.

Free radical formation was significantly accelerated by heating (Fig. 10; III Fig. 3, OBCet=NBG, MVO=OBG). This agrees with the previously reported results of hydroxyl radical formation during the heat-treatments of cellulose in aqueous environment (Robert et al., 2000). Hydrogen peroxide was also readily formed especially at 120°C in NBG, and indications of its consumption by increasing the exposure time were obtained (III, Fig. 4, OBCet=NBG, MVO=OBG). In addition, oxidation of the native β-glucan at 120°C was evident, since the carbonyl groups were formed in significant amounts along the chain (V, Table 2). Thus, these results suggest that Fenton-mediated oxidation contributed to the thermal degradation of the native β-glucan at the temperature of 120°C. Hydroxyl radical formation in the heating of wet cellulose (260°C) was also reported to be hydrogen peroxide mediated (Robert et al., 2000), but in wort the hydroxyl and alkoxyl radical formation (50°C) was not entirely explained by the occurrence of hydrogen peroxide (Frederiksen et al., 2008). In the solution of pure β-glucan (OBG), hydroxyl radicals were formed in significant amounts at 95°C, but the hydrogen peroxide and carbonyl group formation were insignificant. Thus, the role of oxidative cleavage remains somewhat unclear in the case of the slight viscosity and molar mass decrease of the purified β-glucan during the heat-treatments (III, Table 2, MVO=OBG).

The addition of ferrous ions accelerated the free radical formation (Fig. 10), similarly to the previous results with beer (Uschida and Ono, 1996) and wort (Frederiksen et al., 2008). The formation of the carbonyl groups along the β-glucan chain and chain scission of β-glucan was also significantly higher in OBG containing the metallic ions than without (V, Table 2). This may be explained by various oxidation pathways in addition to the Fenton reaction (Reaction 1). The complex iron autoxidation chemistry includes complexation with molecular oxygen and the formation of ferryl and perferryl ions as explained in section 2.3.1.1. These ions may oxidize organic matter by radical and non-radical pathways (Barbusinki, 2009). In the NBG solution, the free ferrous ions may in addition complex with chelates such as proteins, increasing the redox-potential of iron (Welch et al., 2001). By extending the exposure time of OBG at 120°C
Discussion

from 15 minutes to 30 minutes, the number of carbonyl groups decreased (Fig. 11; V, Table 2). This indicates the progression of the oxidative reactions to carboxyls and further oxidation products (Potthast et al., 2006). For example, the high molar mass structures formed at the autoclaving temperature with ferrous ions may consume the carbonyl group for the cross-links, as will be discussed in section 6.5.

6.3 HOMOGENISATION INDUCED DEGRADATION OF β-GLUCAN

High-pressure homogenisation treatments effectively degraded β-glucan, obtained as a steep decrease in the viscosity of NBG and the molar mass of β-glucan. The degradation of β-glucan during homogenisation also displayed a linear relationship between the reciprocal of the molar mass and the degradation rate, which corresponded with rate of ascorbic acid induced degradation and thermal degradation obtained in the present study.

In the studied pressure range of the traditional high pressure valve homogeniser (HPVH) and of the microfluidizer (MF), the degradation of β-glucan had linear relationship with the homogenising pressure ($P$) and molar mass ($M_w$) decrease, being $P \sim M_w^{-0.4}$ and $P \sim M_w^{-0.5}$, respectively (Fig. 13; IV, Fig. 4). The relationship of $P \sim M_w^{-0.7}$ was recorded for methylcellulose degradation in a pilot HPVH at significantly higher homogenisation pressures (Floury et al., 2004). Generally in the emulsion engineering, the MF-technique is considered as more efficient than the traditional HPVH techniques in creating emulsions with small droplets (Stang, et al., 2001, Pinnamaneni et al., 2002; McClements, 2005). The present study suggests the same trend for polysaccharide degradation (Fig. 13). In addition, the high pressure homogenisation decreased the polydispersity of β-glucan (Fig. 14), which was previously demonstrated by Buccholz et al. (2004) as a result of the high velocity of polymers in capillaries. The efficient and pressure-controlled degradation, resulting in a narrow molar mass distribution, makes high pressure homogenisation (microfluidizing) a highly interesting technique for modifying β-glucan to enhance the stability of its solutions.

The degradation forces during the high pressure homogenisation are the main cause of degradation (Stang et al., 2001; McClements, 2005). In addition to these, cavitation also occurs in the high pressure homogenisers, which can cause the oxidation of organic matter (Stang et al., 2001; Gogate and Kabadi, 2009). Oxidation of the functional groups of β-glucan was obtained in the present study, as the carbonyl groups were estimated to occur at concentrations of 5-10 μmol/g after MF-treatments of NBG and OBG (V, Table 2). The carbonyl groups occurred in the low molar mass species with large number of new reducing ends in OBG, but in NBG the carbonyl groups were distributed throughout the whole molar mass range (V, Fig. 4). This indicates that β-glucan was slightly oxidized in the homogenisation treatments, and agrees with the results of Lander et al. (2000), who observed hydroxyl radical formation alongside polysaccharide degradation.
Discussion

Since the oxidation degree appeared to be low and the degradation degree was high, the hydrolysis due to the mechanical shear forces was evidently the predominant degradation mechanism. However, the carbonyl group formation along the polysaccharide chain of a water-soluble polysaccharide was demonstrated for the first time in the present study, and may have a role in modifying the properties of the β-glucan molecule as indicated by the formation of the compact clusters (discussed in section 6.5).

6.4 EFFECT OF THE CO-EXTRACTED COMPOUNDS ON THE OXIDATION

The chemical composition of the solution had a considerable effect on the rate of ascorbic acid induced cleavage and thermal degradation. In both cases, the native β-glucan in NBG degraded more intensively than the highly purified β-glucan in OBG (Figs. 5B and 10). Similarly, the formation of the reactive oxygen species was more significant in the NBG solution than in OBG (Figs. 7 and 10; III, Figs. 3 and 4). In addition, the functional groups of β-glucan in NBG were detectable oxidized by the autoclaving treatments (V, Table 2), while the quantity of carbonyls in OBG was not recognized (V, Table 2). Due to the different reaction times, the ascorbic acid induced carbonyl group formation in NBG and OBG cannot be compared. The homogenisation induced degradation was similar for both solution materials, since the degradation was predominantly due to mechanical forces, which are dependent on the technical properties of the homogeniser.

The differences between these solutions were first of all the purification degree, but also the molar mass, β-glucan concentration and flow properties. The high original molar mass of polysaccharide may increase the degradation rate of water-soluble polysaccharides as shown for instance for κ-carragenans and agarose in ultrasonic radiation (Lii et al., 1999). Thus, the clear difference in the molar mass of the purified and native β-glucan may have been one factor behind the degradation activity. Secondly, as above-mentioned, Bradley et al., (1989) found guar gum to be more susceptible to thermal degradation in concentrated or semi-dilute solutions (c>c*), where the polysaccharides are entangled. The OBG and NBG solutions were both semi-dilute solutions with entangled β-glucan molecules, since the flow behaviours of both solutions were shear thinning. The shear thinning behaviour of NBG was more pronounced than that of OBG with a clear linear phase at the beginning of the flow curves. Since the shear thinning behaviour was fully reversible, the higher susceptibility of NBG to the flow rates indicates a higher degree of entanglements. According to Bradley et al. (1989), this may increase the susceptibility of β-glucan in NBG to the thermal treatments.

The higher rate of free radical formation in NBG than in OBG resulting from heating, the addition of ferrous ion (III, Fig. 3), and the addition of ascorbic acid (Fig. 7) suggests that the higher degradation rate of β-glucan in NBG is due to the
higher rate of oxidation reactions in NBG, rather than the higher susceptibility of the native β-glucan molecule to radical attack when compared with the purified β-glucan. The co-extracted compounds of NBG and their association with β-glucan may have a key role on the degradation rate by the oxidative cleavage. For example, transition metals may associate with β-glucan (Platt and Clydenscale, 1984). In the present study, iron was found to occur at higher concentration in OBG than in NBG (Table 4) and does not, therefore straightforwardly explain the higher rate of oxidative degradation of the native β-glucan. In contrast, the amount of copper, a more efficient catalyst of the Fenton-type reaction than iron, was higher in the NBG than in OBG (Table 4). Persson et al. (1991) examined the binding of Cu²⁺, Zn²⁺ and Cd²⁺ by soluble fibre of oat, barley and rye and found that pure β-glucans did not complex metal ions but the chelating agents associated with them, were responsible for the binding. In other hand, Welch et al. (2000) suggested that when iron complexes with chelates or proteins, its oxidation capacity in aqueous biological conditions significantly increased. In addition, a negatively charged nature of oat β-glucan has been observed and suggested to be due to proteins (Vårum et al., 1992) or phosphorus compounds (Ghotra et al., 2007). These facts may increase the rate of hydroxyl radical attack, since the solvated radicals are known to more readily attack the charged groups of the polysaccharide (Gilbert et al., 1984), or through the increase in hydroxyl radical production via the complexation of the catalysing metals. Ghotra et al. (2007) demonstrated that the negatively charged phosphorus compounds were associated with the C-6 of the glucose residue of the highly purified oat β-glucan chain, and that part of it consistend of the natural chelator of oats, phytic acid (phytate). Phytic acid is considered as an antioxidant towards Fenton reaction (Graf et al., 1987), but the role of chelates seems to be somewhat controversial (Buettner and Jurkiewicz, 1996; Burkitt and Gilbert, 1990). In the present study, phosphorus, assumingly phytate, occurred in high concentrations in NBG (Table 4), and the addition of phytic acid accelerated the viscosity loss of NBG with and without ascorbic acid (Fig. 6). According to this, phytic acid (phytate) alongside proteins is a co-extracted compound, which potentially have an important role in β-glucan oxidation and the chain cleavage.

The role of co-extracted constituents and molecules associated with β-glucan in the rate of thermal degradation was further tested by heating a purified NBG (NBGp). The molar mass of β-glucan did not considerably decrease after the purification step, while without the purification, the molar mass significantly decreased and tcarbonyls were formed (Fig. 12). Similarly, the simultaneous formation of the carbonyl groups was significantly high in the unpurified β-glucan, but nonsignificant after purification. This clear inhibitory effect of the purification without changes in the original molar mass, β-glucan concentration or molar mass analysis method strongly supports the hypothesis of the boosting effect of the co-extracted compounds on thermal oxidation.
6.5 CROSS-LINKING OF TREATED β-GLUCAN

High molar mass β-glucan species with a compact conformation were observed after ascorbic acid, heat and homogenisation treatments in OBG (V, Fig. 2a). Typically these findings related to the extent of degradation of the β-glucan molecule or in other words, with possible high degree of oxidation. Interestingly, when treating OBG at a sterilisation temperature for 30 min with added ferrous ions, the appearance of a second molar mass population was observed. This second population had a significantly higher weight average molar mass than the original population, while the first molar mass population had a significantly lower $M_w$ than the original (V, Fig. 2b). This indicates a degradation process, after which the degraded molecules form molecular complexes.

The compact nature of the high molar mass species suggests the formation of aggregates and clusters, since the exponent of the conformation plot <0.5 is a clear indication of branching or cluster formation (Ioan et al, 2000). The plot value < 0.333 indicates hard spheres, and the plots approaching zero obtained in this study (V, Fig. 2a) most probably indicate a side-to-side aggregation, when the molar mass of the cluster rapidly increases (Burchard, 2003). The clustering may be due to the hydrogen bonded side-to-side aggregation reported for β-glucans with low molar mass (Grimm et al., 1996, Böhm and Kulicke, 1999b). However, since the analysis was performed in an organic solvent, the hydrogen bonds may have been eliminated, and the formation of the new structures may indicate covalent bonding. Potthast et al. (2007) demonstrated the formation of highly compact and ordered regions of cellulose by periodate oxidation. This was explained by hemiacetal cross-linking of the oxidatively degraded cellulose chains and the hydroxyl groups of the glucose residues. Similarly, the number of carbonyl groups of the pullulan chain affected a molar mass increase rather than a decrease, as the thermal exposure time was increased (Strilc et al., 2003). Thus, this type of cross-linking of the oxidized molecules is also a highly relevant explanation for processed β-glucan in aqueous solutions.

6.6 ASPECTS OF THE MANAGEMENT OF β-GLUCAN IN LIQUID FOODS

Acid hydrolysis of β-glucan as a non-enzymatic degradation mechanism has been suggested to play a role in the manufacture of juice-based beverages (Åman et al., 2004), since the molar mass of oat β-glucan considerably decreases during the manufacture of the drinkable products (Table 2, Åman et al., 2004; Lazaridou et al., 2007; Naumann et al., 2006; Björklund et al., 2006). Part of the degradation is a desired enzymatic hydrolysis process, but also uncontrolled degradation is evident. The processing of beverages usually includes a heating step to pasteurize or sterilize the products, which may lead to acid hydrolysis in the presence of the juice-based carboxylic acids. The heating step in pasteurization and sterilization commonly lasts few seconds, followed by
effective cooling, and the pH of regular juices is approximately 3–3.5, but probably even higher in cereal-based beverages.

In the present study, neither the common carboxylic acids (malic and citric) nor HCl considerably reduced the viscosity of NBG with or without a heating step under slightly acidic conditions (II, Fig. 3). This agrees with the previous results obtained with barley β-glucan solutions, for which viscosity was not significantly affected by 5 minutes of treatment at 82.5 °C (Vaikousi and Bilianderis, 2005). As illustrated in Figure 3 (II), a slight decrease in the viscosity of NBG following the addition of malic acid and HCl (pH control) was obtained after the heating step. However, a similar viscosity decrease was also obtained in the extract with a pH of 7, and may thus reflect alterations in the conformation of the molecule or molecule aggregates, or alternative degradation pathways rather than hydrolysis of the backbone. Based on the slight effect of other carboxylic acids than ascorbic acid, and the formation of hydroxyl radicals at 95 °C, the role of acid hydrolysis in the degradation of β-glucan during the processing of liquid foods is negligible, and the more likely non-enzymatic degradation mechanism is oxidative cleavage. Depending on the manufacturing process, hydrolysis induced by mechanical forces may also have a significant role in the degradation (Fig. 13).

The oxidation reactions in foods are generally managed by antioxidants, chelating agents and oxygen depletion. In the case of the oxidation of watersoluble polysaccharides in food-related conditions, antioxidants seem to have a controversial role. Not only did the ascorbic acid have a high pro-oxidant intensity, but so too did sulphite (III, Table 3), as previously reported for starch (Paterson et al., 1997). However, when ascorbic acid and sulphite were used together, the thermal degradation of β-glucan in presence of ferrous ions was significantly slowed down (III, Table 3). The viscosity remaining after 5 minutes of treatment at 120 °C was 90% in OBG and 35% in NBG in presence of ascorbic acid and sulphite, while the respective figures without the additives were 70% and 3%. Hill and Gray (1999) found a similar effect by combining propyl gallate and sulphite to stabilize the viscosity of galactoglucomannan solutions during autoclaving (120 °C) treatments. The pro-oxidative nature of sulphite has often been associated with $SO_3^-$ radical formation by hydroxyl radicals. This radical easily reacts with molecular oxygen, producing peroxy sulphate radicals ($SO_5^-$) with a high intensity to oxidize organic matter (Neta and Huie, 1985; Wedzicha, 2000). However, the peroxy sulphate radical also rapidly reacts with ascorbic acid, decaying by itself and producing ascorbyl radicals (Neta and Huie, 1985). Thus, it can be hypothesised that the peroxy sulphate radicals formed by the thermal treatments using dissolved oxygen reacted with ascorbic acid, converting it to the antioxidant form, and thus protected β-glucan from the oxidants instead of oxidizing them. This effect was promising in searching for a way to manage processing-induced oxidative cleavage.

Although Fenton chemistry evidently occurs in vitro, its relevance in vivo has been criticized (Carr and Frei, 1997). The criticism of the relevance of Fenton-
mediated oxidation \textit{in vivo} has often concerned the poor availability of catalytic metal ions in biological conditions. In food systems, several factors such as the food matrix and water activity obviously also affect the reaction rates and the attack sites, and the isolated polysaccharides in water solutions are assumedly more susceptible to oxidation reactions than those in cell walls during dry processes, for instance. However, the evident oxidation of β-glucan even in mild conditions warrants further investigation to reveal the threats and challenges of this new type of mechanism in the degradation and functional modification of cereal β-glucan.

6.7 THE AGGREGATION PHENOMENON AT THE STUDY

Aggregation was considered throughout the study in the interpretation of the real molecular degradation during the investigated processes compared to aggregate dissociation and conformational changes, both of which result in viscosity losses. The shape of the molecule can be determined by the parameter $\rho = R_g/R_h$, which depends on the chain architecture, conformation and polydispersity, but not on the molar mass (Burchard, 1995). For polydisperse random coils, $\rho = 2.05$ in a good solvent and $\rho = 1.73$ in a theta solvent, while for star-branched structures $\rho = 1$ and for a rigid sphere $\rho = 0.7$ (Grimm et al., 1995; Burchard, 1995). The $\rho$-values of 1.5-1.7 were recorded for β-glucan in heat-treated or high pressure homogenised NBG and OBG. This agrees with the value reported for a wheat β-glucan unimer ($\rho=1.7$; Li et al., 2006), and typically reflects a flexible conformation such as a random coil. The value of the shape parameter suggest a relatively low degree of apparent aggregation of β-glucan at the time of the analysis in HPSEC.

The dissociating effect of the HPSEC-conditions, which combines shear flow, temperature and dilution before light scattering detection, was previously suggested by Li et al. (2006), Gomez et al. (1997a) and Vårum et al. (1992). Li et al. (2006) demonstrated that the size of non-aggregates in NaOH measured by light scattering was similar to the HPSEC-results with an aqueous eluent. The same results were obtained here, when the NaOH-treatment was combined with dynamic light scattering in our tentative experiments (Fig. 15). No complete dissociation of the aggregates was obtained by the NaOH-treatment, but two populations were obtained, of which the low molar mass population was consistent with the average size ($R_h$) of β-glucan determined by HPSEC in the the aqueous environment (circles in Fig. 15). According to Li et al. (2006) these preliminary results indicate that β-glucan had a low degree of aggregation during the HPSEC-analysis, and the molar mass values used to determine the extent of the degradation in present study, were therefore reliable.
7 CONCLUSIONS

The present study demonstrated that β-glucan is susceptible to non-enzymatic degradation in aqueous solutions. Oxidative cleavage was discovered to be a relevant process-related degradation mechanism for solubilized β-glucan, since oxidative reactions contributed to the considerable degradation of β-glucan molecule. Simultaneously, oxidized functional groups (carbonyls) were formed along the β-glucan chain during the studied treatments.

The studied treatments, namely ascorbic acid addition, heating and high pressure homogenisation, immediately decreased the viscosity of β-glucan solutions and cleaved the β-glucan chain. The kinetic illustrations indicated a random scission mechanism with a degradation rate mainly between $10^{-4}$-$10^{-6}$ min$^{-1}$. The degradation rate was significantly higher for a native β-glucan in a solution with the co-extracted compounds than for the highly purified β-glucan, which is an important aspect regarding food products.

Ascorbic acid treatment caused the oxidative cleavage of β-glucan, since carbonyl groups were formed along the β-glucan chain simultaneously with the molar mass decrease of β-glucan in solutions containing ascorbic acid. The contribution of the oxidative reactions in β-glucan solutions with added ascorbic acid was demonstrated by hydroxyl radical formation, the consumption of oxygen, as well as the accelerating effect of added ferrous ions and contribution of hydrogen peroxide in the ascorbic acid induced viscosity loss. All these results indicated that oxidative cleavage, in particular Fenton chemistry derived oxidation, had a key role in the ascorbic acid induced degradation of β-glucan in both of the studied β-glucan solutions.

Thermal treatments (120°C) also caused the formation of carbonyl groups consistently with the molecular degradation of the native β-glucan. Hydroxyl radical and hydrogen peroxide formation were significant in the thermal treatments of the β-glucan solution with the co-extracted compounds. Thus, oxidative cleavage was also evident in the thermal treatments of native β-glucan with co-extracted compounds. The highly purified β-glucan was only slightly affected by the treatments at 120°C, and the contribution of oxidative cleavage remained somewhat unclear in this case. However, with a higher concentration of added ferrous ions, this highly purified β-glucan also clearly cleaved during thermal treatments. In general, despite the considerably changes observed in the present study, β-glucan appeared to be relatively resistant to heating when compared to data in literature of other water-soluble polysaccharides.

To protect the thermal treatments induced viscosity loss, antioxidants were tested. Interestingly, the viscosity decrease was slowed down by adding ascorbic
Conclusions

acid together with sulphite. When added individually, also sulphite had a pro-
oxidant effect, but when added together, an antioxidant effect was observed.

High pressure homogenisation slightly induced formation of carbonyl groups
along the β-glucan chain, as was demonstrated for the first time in the present
study. This indicates the contribution of oxidation to the changes in β-glucan
during high pressure homogenisation. Homogenisation caused an intense
degradation of β-glucan, but the carbonyl groups content was small. Thus, the
predominant degradation mechanism was most probably hydrolysis driven by
mechanical shear, although oxidation may contribute to the properties of the
degraded β-glucan. In general, high pressure homogenisation proved to be an
efficient technique for modifying β-glucan, since the degradation was pressure-
controllable and resulted in a narrow molar mass distribution and the treatment
inhibited the formation of compact assemblies during the storage of β-glucan
solutions.

A formation of highly compact high molar mass species, most likely in the form
of compact clusters, was obtained after treatments (ascorbic acid, 120°C+Fe^{2+},
homogenisation). This was most likely due to the covalent cross-linking of the
oxidized and unoxidized functional groups of β-glucan resulting in severe
changes in the properties of β-glucan. Thus, the oxidation process may decrease
the viscosity of the β-glucan solutions not only by the cleavage of the chain but
also by formation of new structures and conformational changes of the oxidized
chain. Overall, this study demonstrated that the managing of the oxidative
cleavage of β-glucan is challenging in food solutions, but suggests, however, that
there are advantages to utilize oxidative reactions for the modification of β-
glucan.

In the future, the oxidation mechanism needs to be further studied focusing on
the reaction pathways and the effect of solution conditions on them. Low molar
mass and polymeric oxidation products after carbonyl group formation state are
important to be examined to understand the threats and advantages of the
oxidation process. In addition, the role of oxidation in real food systems and
matrices is necessary to understand. However, the knowledge conceived in the
present work revealed important questions, and will as such improve the
management of β-glucan modification for novel products.
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References


References


References


References


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Reetta Kivelä (Ansaharju) was born in February, 1977, in Hämeenlinna, Finland. After completing her upper secondary school, she first studied chemistry in Univeristy of Helsinki completing the cum laude in chemistry and approbatur in physics in 1998. She then started food chemistry studies in the Department of Applied Chemistry and Microbiology in Univeristy of Helsinki. In 2003, she obtained her MS degree in food sciences, with food chemistry as the major topic, the master thesis was titled as *Synthesis of the 5α,6α - and 5β,6β-epoxides and 3β,5α,6β-triol of stigmasterol*. During 2003-2004, she worked at the Food and Environmental Laboratory of Häme area as an associate chemist. From August 2004, she was at Forssan Liha ja Säilyke Oy (Atria Oyj) first as a Project Technologist, and then until 2007 as a Project Manager being responsible for process development and quality tasks. In 2007, she returned to PhD studies in University of Helsinki in the cereal technology group. She visited Boku, Vienna studing the process-related oxidation of β-glucan under supervision of prof. Antje Potthast, and has had fruitful collaboration with ETH, Zurich in addition to the domestic facilities. The courses of PhD studies she took in Helsinki and Wageningen, the Netherlands. In 2010, she won the third place in the *AACC Intl. Best Student Research Paper Competition*. In addition to the topics of the present work, she has worked with gastric mucin β-glucan interactions, oat based emulsions and other β-glucan enriched applications in domestic and international collaboration projects including co-supervising of master theses. These research projects have resulted in 9 scientific articles and a book chapter. Currently she continues her career at the Fazer Group as a Research Technologist.