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ROLE OF LIPIDS AND PHYTATE IN OXIDATIVE STABILITY OF CEREAL BETA-GLUCAN

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ACADEMIC DISSERTATION

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ABSTRACT

β-Glucan (β-(1→3),(1→4)-D-glucan) is the major non-starch polysaccharide in oats and barley, and it is well-known due to its physiological and technological benefits, which are related to the ability to increase the luminal or solution viscosity. However, cereal β-glucan is susceptible to degradation during processing and storage, which may deteriorate the product stability and/or β-glucan functionality. Oxidative degradation of β-glucan has been shown in aqueous systems. In multi-phased food systems containing lipids, the oxidative stability of β-glucan has not yet been studied. In these systems, lipid oxidation is a major source of radicals which can cause co-oxidation of other components. The aim of the study was to understand the oxidative stability of cereal β-glucan during lipid oxidation, and the simultaneous role of β-glucan in retarding lipid oxidation. Furthermore, the study investigated the contribution of the residual phytate in β-glucan to the oxidative stability of β-glucan.

Results showed that lipid oxidation induced significant degradation of β-glucan in an oil-in-water emulsion model, as evidenced by a decrease in viscosity and decrease in molecular weight of β-glucan. The increase in the degree of oil oxidation, the concentration of transition metal or the storage temperature caused a greater extent of β-glucan degradation. Simultaneously, a retardation of lipid oxidation was observed in the emulsions containing β-glucan. The mechanism was further investigated by using purified oat and barley β-glucans with different molecular weights. Initially, it seemed that the retardation of lipid oxidation was determined by the β-glucan source and the molecular weight. However, the retardation was found to correlate with the content of residual phytate in the β-glucan samples. When the phytate was removed, the retardation of lipid oxidation by β-glucan disappeared regardless of the β-glucan source and molecular weight. Therefore, the residual phytate in the β-glucan samples, instead of β-glucan structural features, played a major role in the retardation of lipid oxidation. The study further proved that the residual phytate protected the β-glucan from oxidative degradation. Under oxidative conditions, oat β-glucan containing a higher amount of phytate was more stable than barley β-glucan containing less phytate. The oat β-glucan became as vulnerable as barley β-glucan to the oxidative degradation when the residual phytate was removed.

The addition of phytic acid also retarded the degradation of β-glucan, which was affected by the molar ratio of phytic acid to iron and the presence of competitors such as ascorbic acid. The studies indicate that oxidized lipids and co-passengers of β-glucan can influence the oxidative stability of β-glucan, and consequently influence its technological and physiological functionality.

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Yu-Jie Wang

王玉洁

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications. In addition, previously unpublished data are presented.

I Wang, Y.J., Mäkelä, N., Maina, N.H., Lampi, A., & Sontag-Strohm, T. 2016. Lipid oxidation induced oxidative degradation of cereal β-glucan. *Food Chemistry,* 197, Part B: 1324-1330.

II Wang, Y.J., Maina, N.H., Ekholm, P., Lampi, A.M. & Sontag-Strohm, T. 2017. Retardation of oxidation by residual phytate in purified cereal β-glucans. *Food Hydrocolloids,* 66: 161-167.

III Wang, Y.J., Zhan, R., Sontag-Strohm, T. & Maina N.H. 2017. The protective role of phytate in the oxidative degradation of cereal β-glucan. *Carbohydrate Polymers*, 169: 220-226.

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Contribution of the author to publication I to III:

I Yu-Jie Wang planned the study together with the other authors. She performed most of the experiments, and shared the responsibility for interpreting the results and writing the article together with the other authors. She was the corresponding author of the paper. This article was also included in the dissertation of Noora Mäkelä.

II, III Yu-Jie Wang planned the study together with the other authors. She had the main responsibility for the experiments and interpreting the results. She was the corresponding author of the papers.

ABBREVIATIONS

AA	Ascorbic acid
β-Glucan	Cereal β-(1→3),(1→4)-D-glucan
BBG-H	Commercial barley β-glucan with high molecular weight
BBG-L	Commercial barley β-glucan with low molecular weight
DHA	Dehydroascorbate
DMSO	Dimethyl sulfoxide
DP	Degree of polymerization
EDTA	Ethylenediaminetetraacetic acid
HML	Highly oxidized methyl linoleate
HPSEC	High pressure size exclusion chromatography
HRO	Highly oxidized rapeseed oil
ICP-OES	Inductively coupled plasma optical emission spectrometry
IP1-6	Inositol mono-, bis-, tris-, tetra-, penta-, hexa-phosphate
$L^{\bullet}$	Lipid alkyl radical
$LO^{\bullet}$	Lipid alkoxyl radical
$LOO^{\bullet}$	Lipid peroxyl radical
LOOH	Lipid hydroperoxide
ML	Methyl linoleate
MML	Mildly oxidized methyl linoleate
M_w	Weight-average molecular weight
M_n	Number-average molecular weight
M_p	Peak molecular weight
NRO	Non-oxidized rapeseed oil
OBG-H	Commercial oat β-glucan with high molecular weight
OBG-L	Commercial oat β-glucan with low molecular weight
P	Phosphorus
PA	Phytic acid
PV	Peroxide value
RNS	Reactive nitrogen species
RO	Purified rapeseed oil
ROS	Reactive oxygen species
UV	Ultraviolet

1 INTRODUCTION

Cereal β-(1→3),(1→4)-D-glucan is a partially water-soluble non-starch polysaccharide that can be found in high levels in oats and barley. It is mainly located in the cell wall of the endosperm and aleurone layers (Cui and Wood, 2000). It is used in foods as fiber for nutritional functionality and as hydrocolloids for technological functionality. In food products, oat bran and bran concentrates or whole grain barley with high content of β-glucan are commonly used. Oat and barley β-glucan have gained much attention due to their well-evidenced health benefits. Health claims including the reduction of the risk for heart diseases, lowering of blood cholesterol, and management of blood glucose levels have been approved by authorities such as the US Food and Drug Administration (FDA 1997, 2005) and the European Food Safety Administration (EFSA 2009, 2010a,b, 2011a,b,c). These physiological benefits of cereal β-glucan are conferred by the combination of several mechanisms, involving, but are not limited to, an increase in the digesta viscosity within the lumen of the gastrointestinal tract, interaction with starch and other nutrients, interference with the activity of gut enzymes, and an increase in the excretion of bile salts, as stated by Wang and Ellis (2014).

The viscosity and molecular weight of cereal β-glucan have been suggested as the most important factors in lowering cholesterol and attenuation of blood glucose by β-glucan. However, cereal β-glucan is susceptible to degradation by e.g. enzymes, acids, heat and mechanical forces, which deteriorate its stability and functionality. In addition, oxidative degradation of cereal β-glucan by hydroxyl radicals produced from the Fenton reaction or ascorbic acid and transition metals has been shown in aqueous systems (Kivelä et al., 2009; Faure et al., 2013; Mäkelä et al., 2015). In multi-phased systems, radicals from lipid oxidation have been shown to cause co-oxidation of polymers such as protein, starch and other components (Schaich, 2008). Whether oxidized lipids can cause degradation of β-glucan has not been studied.

Some polysaccharides have been shown to exert antioxidant activity with mechanisms involving viscosity enhancement which reduces the mobility of reagents, transition metal binding and free radical scavenging (Matsumura et al., 2003; Shimada et al., 1992; Shimada et al., 1996). However, the studied polysaccharides contain significant amounts of impurities which may have a large influence on the antioxidant effect of the polysaccharides. For example, phenolic compounds can be bound or entrapped mostly by polysaccharides that are not water extractable, and these compounds can contribute to the antioxidant effect (Rao and Muralikrishna,

2004). The presence of phytate in the soluble fiber fraction of oat grain, containing mostly β-glucan, has been noticed earlier (Frølich and Nyman, 1988). Kivelä (2011) has shown that the phytate content in β-glucan extracts obtained from oat bran concentrate was as high as 6%. Compared to barley, the high amount of phytate in oat products is possibly due to the common thermal treatment (e.g. kilning) of oats which leads to inactivation of endogenous enzymes, including phytase. It has been indicated that oat β-glucan is more stable than barley β-glucan under oxidation (Faure et al., 2015). Phytate is known as an antioxidant, therefore whether phytate in the β-glucan sample contributes to the oxidative stability of β-glucan need to be investigated.

In this thesis, the literature review offers an overview of cereal β-glucan and phytate in cereal fibers. Lipid oxidation and its co-oxidation of other molecules, and the role of polysaccharides in emulsions are also reviewed. The experimental work investigated the degradation of β-glucan induced by lipid oxidation using an oil-in-water emulsion model, and the role of phytate in the oxidative stability of β-glucan. This study aimed to provide an understanding of the oxidative stability of β-glucan in complex food systems which contains pro-oxidants such as lipids and antioxidants such as phytate.

2 REVIEW OF THE LITERATURE

2.1 Cereal β-(1→3),(1→4)-D-glucan

2.1.1 Sources and distribution

β-Glucan can be found in the cell walls of cereals such as oats, barley, rye and wheat. Compared to other cereals, oats and barley contain relatively higher levels of β-glucan, which are 3-7% and 3-11%, respectively (Cui and Wood, 2000). The levels are influenced by genetic and environmental factors (e.g. variety, growing time and temperature) (Saastamoinen et al., 1992). β-Glucan is the primary component of soluble dietary fiber in both oats and barley. Most β-glucan is located in the inner layer of the cell walls which is enclosed by an insoluble cellulosic and hemicellulosic outer layer (Miller and Fulcher, 2011). In oats, β-glucan is mainly concentrated in the bran fractions, with the highest concentration in the thick cell walls of the subaleurone layer and also in the starchy endosperm tissue attached to the aleurone layer (Figure 1). On the contrary, in most barley grains, β-glucan is evenly distributed throughout the aleurone and endosperm (Miller and Fulcher, 1994).

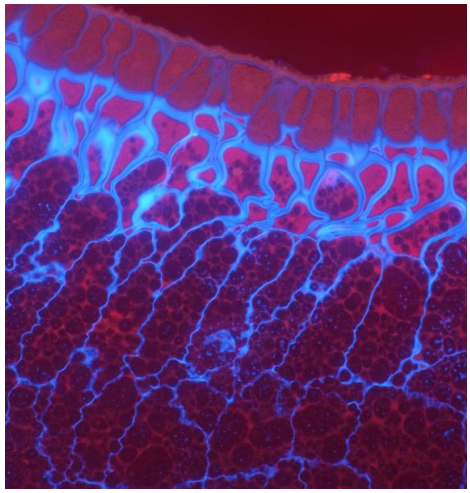

Figure. 1 Scanning microspectrofluorometry of β-Glucan in the cell wall of oat groats stained (light blue) with Calcofluor. (Courtesy of Shea Miller, Agriculture and Agri-Food Canada)

Oat and barley β-glucan can be enriched by dry milling, sieving and air classification (Izydorczyk et al., 2000), or produced in high concentrations up to 95% by wet extraction and purification (Bhatty, 1995). For example, the content of β-glucan in oat bran fractions, oat bran concentrates and β-glucan isolates can be 6-8%, 15-40%, and up to 80%, respectively (Kaukovirta-Norja et al., 2008; Lehtinen et al., 2009; Kivelä, 2011). β-Glucan isolates with high purity are expensive and they are mostly used in research. In food products, bran and bran concentrates from oats or whole grain barley with high β-glucan content are commonly added.

2.1.2 Physicochemical properties

β-Glucans from cereals are linear polysaccharides consisting of β-D-glucopyranosyl units linked by either of β-(1→3) or β-(1→4) linkages (Figure 2). About 90% of the polysaccharide chain is comprised of cellotriosyl (DP3) and cellotetraosyl (DP4) units that are linked through β-(1→3) linkages, and with the rest being longer cellulosic segments (Wang and Ellis, 2014). The DP3 and DP4 units are randomly arranged along the chain (Staudte et al., 1983). Unlike cellulose containing only β-(1→4) linked glucopyranosyl residues, the β-(1→3) linkages in β-(1→3),(1→4)-glucan break up the regular structure and extend the chain with limited capacity to align with other β-(1→3),(1→4)-glucan chains, making it more soluble in water (Figure 2). The physiochemical properties of β-glucan are affected by the ratio of DP3 and DP4 linkages, the amount of long cellulose-like fragments, and the molecular size (Lazaridou and Biliaderis, 2007).

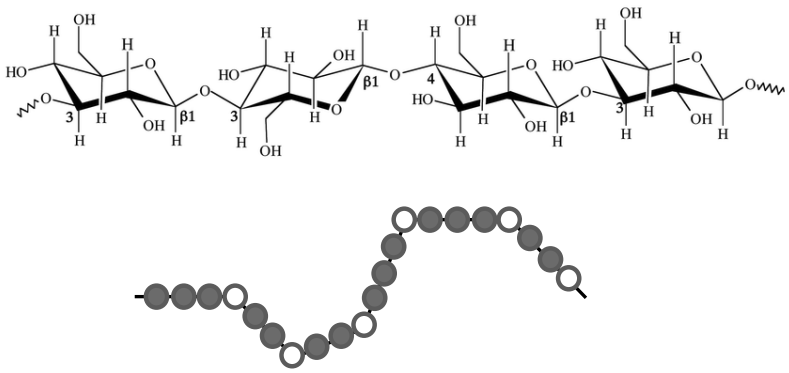

Figure 2. Chemical structure (above) and schematical chain (below) of cereal β-(1→3),(1→4)-glucan. (Modified from Burton et al., 2010).

2.1.2.1 Molecular weight and conformation

The molecular weight and conformation of polysaccharides are important determinants of their physical properties such as solubility, viscosity and gelation properties (Wang and Ellis, 2014). Natural polysaccharides have a polydiperse molecular weight distribution, with an averaged value based on the measurement and calculation methods. For β-glucan, the peak molecular weight (M_p), the weight-average molecular weight (M_w) and the number-average molecular weight (M_n) are most often used. The molecular weight of cereal β-glucans that has been reported ranges from 20 000 to 3100 000 g/mol, depending on the variety, extraction protocols and the analytical methods used in the determination of the value (Izydorczyk et al., 2000; Cui and Wood, 2000; Lazaridou and Biliaderis, 2007). Single-stranded β-glucan is a partially stiff and worm-like expanded coil in aqueous solution. Grimm et al. (1995) have stated that β-glucan tends to aggregate in aqueous solution possibly by intermolecular hydrogen bonding between hydroxyl groups (Grimm et al., 1995). Low molecular weight β-glucans in solution have been shown to exhibit fairly rapid aggregation (Lazaridou et al., 2003). However, Mäkelä et al. (2015) have recently reported that no severe aggregation occurred in barley β-glucan within one week and significant aggregates were found only in oxidized β-glucan which contained an internal carbonyl group formed through oxidation (Mäkelä et al., 2017).

Stability of β-glucan chain

Cereal endogenous enzymes (e.g. from wheat kernel) or microbial enzymes (e.g. lichenase) that are contaminants in the material may hydrolyze the β-glucan, which lowers the molecular weight especially in wet processing (Åman et al., 2004; Vatandoust, 2012). Therefore, in order to maintain the molecular weight, inactivation of these enzymes using heat treatment or boiling in aqueous ethanol is required. Under highly acidic conditions and high temperatures (120°C), total hydrolysis of β-glucan can occur (Johansson et al., 2006). Mechanical forces applied in many processes may degrade β-glucan molecules. Kivelä et al. (2010) have shown depolymerization of β-glucan after homogenization with microfluidizer (pressure 100 MPa; 10 min), with the molar mass deceasing from 1 440 000 to 130 000 g/dl. Beer et al. (1996) have shown that different isolation methods in the production of oat gum affected the molecular weight and solution viscosity of β-glucan. In their study, the dialysis produced a highly viscous β-glucan solution, whereas ultrafiltration and

alcoholic precipitation produced β-glucan extracts with lower viscosity. The high shear forces produced during ultrafiltration and decanting centrifugation after ethanol precipitation may degrade β-glucan causing low viscosity and molecular weight (Beer et al., 1996).

2.1.2.2 Solubility

The solubility of a polysaccharide is usually expressed as the percentage of the dissolved fraction relative to the total amount of the polysaccharide in the original solid matrix under specific extraction conditions (Wang and Ellis, 2014). For the isolated and purified β-glucan material, solubility refers to the extent of dissolution. For the β-glucan within the food matrix, the term extractability is used, which refers to the percentage of β-glucan that can be extracted. It is more meaningful to compare the solubility of different β-glucans under the same conditions. The solubility of β-glucan can be affected by molecular weight. Tosh et al., (2008) have shown that the solubility of β-glucan increased from 44 to 57% while the molecular weight of β-glucan decreased from 2 200 000 to 400 000 Da. Further reduction of molecular weight to 120 000 Da, however decreased the solubility to 26%. This poor solubility at a low molecular weight was suggested to be caused by insoluble aggregates formed by stronger self-association of the depolymerized β-glucan (Tosh et al., 2008).

The main structural difference between oat and barley β-glucan is the DP3:DP4 ratio, which is higher for barley than for oat (Wood et al., 1994; Johansson, 2006). Studies have reported that oat β-glucan has better solubility than barley β-glucan due to the lower DP3:DP4 ratio in oat β-glucan (Wood 1993). Johansson (2006) has shown that barley β-glucan had a higher tendency towards gelation than oat β-glucan, which was attributed to the higher DP3:DP4 ratio of barley β-glucan. With several Canadian barley genotypes, Cui et al. (2000) found that β-glucans in the endosperm cell walls had lower DP3:DP4 ratios than β-glucans in the aleurone and pericarp tissues. Consistently, compared to the β-glucans in the thick subaleurone cell walls, better solubility of β-glucans in the thinner inner endosperm cell walls has been reported (Autio et al., 1992). In addition to the lower DP3:DP4 ratio of β-glucan in endosperm, the cell walls in the inner endosperm are thinner than in the aleurone and subaleurone layers, therefore β-glucan can be released easier in endosperm (Miller and Fulcher, 2011; Wang and Ellis, 2014). Beer et al. (1997) have found that β-glucan in the milled oat groats is more easily extracted than that in oat bran.

The extractability of β-glucan can be affected by food processing, the structure of the food as well as the other components in the food matrix. For example, freezing has been found to reduce the extractability of β-glucan in oat muffins (Beer et al., 1997). Grundy et al. (2017), have shown that raw oat flakes released the β-glucan more gradually than raw oat flours due to the limited particle size and surface area of the flakes. The addition of protease and esterase during extraction has been shown to improve the extractability of β-glucans from barley (Izydorczyk et al., 2000).

2.1.2.3 Viscosity

The viscosity of a polysaccharide solution is dependent on the polymer concentration, molecular weight, chain conformation, intermolecular interactions as well as solvent and measurement conditions e.g. shear rate and temperature (Lazaridou et al., 2007; Wang and Ellis, 2014). β-Glucans are able to form viscous solutions at relatively low concentrations, and the viscosity increases with the increase of β-glucan concentration and molecular weight. At lower concentrations, the viscosity is independent on shear rate and the β-glucan solution behaves like a Newtonian fluid (Figure 3). At the critical concentration, the β-glucan molecule chains start to overlap and entangle (Ren et al., 2003). At higher concentrations, the β-glucan solution is pseudoplastic (shear thinning). The decrease in viscosity with the changing shear rate results from alignment of molecules in the direction of shear or breakdown of the entanglements of molecules during shear (Morris et al., 1981). The critical concentration of the polymers varies depending on the molecular weight distribution. The critical concentration for barley β-glucan with a molecular weight of 50 000 g/mol and 375 000 g/mol has been shown to be 2% and 0.5%, respectively (Böhm and Kulicke, 1999). The low molecular weight β-glucan may form soft gels at high concentrations (Doublier and Wood, 1995).

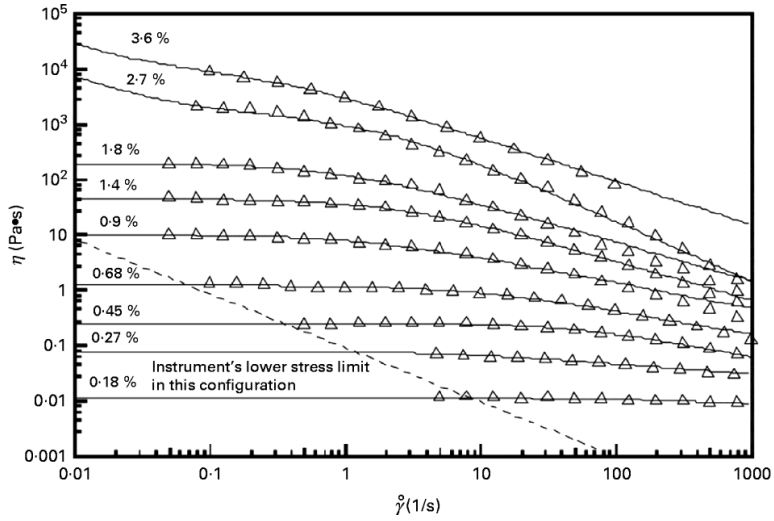

Figure 3. Flow curves (apparent viscosity (η) *vs* shear rate ($\dot{\gamma}$)) for solutions containing different concentrations of oat β-glucan (the intrinsic viscosity of the β-glucan was 9.3 dl/g). Adapted from Ren *et al.,* 2003, copyrights Elsevier Science Ltd.

2.1.3 Health benefits

The health benefits of dietary fiber have been demonstrated several decades ago. Cereal fractions containing β-glucan have been extensively used in various food products in order to improve the nutritional and technological value. Among various types of dietary fibers, cereal β-glucan is one of the few which has health claims concerning cholesterol lowering and attenuation of blood glucose approved by FDA and EFSA (FDA 1997, 2005; EFSA 2010b, 2011a,b,c). The health effects of water-soluble β-glucan are attributed to the ability of β-glucan to form highly viscous solutions in the upper digestive tract. For example, the increase in luminal viscosity reduces or delays the absorption and diffusion of the nutrients such as glucose, lipids and bile acids (Vahouny et al., 1980; Mälkki and Virtanen, 2001). The viscous β-glucan solution acts as a barrier, limiting the contact between the digestive enzymes and the food substrates. Studies have shown that β-glucan could retard starch digestion and glycemic response, and the effect was enhanced by an increase in viscosity (Mäkeläinen et al., 2007; Regand et al., 2011). The viscosity of β-glucan may hinder the absorption of bile acids or cholesterol directly. The hypocholesterolemic action of certain dietary fibers has been linked to bile acid binding (Guillon and Champ, 2000) but the mechanisms of bile acid binding to fibers are not fully understood.

The viscosity and molecular weight of cereal β-glucan seem to be the most important factors in cholesterol lowering and blood glucose management by β-glucan. However, some studies have shown that low molecular weight β-glucan can have the same effect. For example, Bae et al., (2010) have reported that the enzymatically hydrolyzed oat β-glucan could improve the cholesterol lowering effect more than native β-glucan. The controversial results indicate that other mechanisms may exist. Anaerobic bacterial fermentation of soluble fiber such as β-glucan produces short-chain fatty acids which are important in balancing the colonic microbiota and improving glucose metabolism (Guillon and Champ, 2000).

2.2 Oxidative degradation of cereal β-glucan

In biological systems, polysaccharides can be degraded by reactive oxygen species (ROS) or reactive nitrogen species (RNS) originating from either exogenous or endogenous sources (Kohen and Nyska, 2002). ROS/RNS includes free radicals containing one unpaired electron ($O_2^{\bullet -}$, $^{\bullet}OH$, $ROO^{\bullet}$, $RO^{\bullet}$, $NO^{\bullet}$) and non-radicals such as hydrogen peroxides. Depolymerization of polysaccharides by ROS/RNS is important in cell wall loosening and it provides an alternative way in the catabolism of polysaccharides in vivo during plant growth (seed germination, elongation growth, and fruit ripening) which requires structural changes in the cell walls (Muller et al., 2009; Duan and Kasper, 2011). Studies have found oxidation of cell wall polysaccharides, including cereal β-glucan, in the process of cell-wall loosening during the growing phase of plants (Muller et al., 2009). In food processing, oxidative degradation of polysaccharides may occur, which is indicated by some studies with model systems (Kivelä et al, 2009; 2011). Oxidative degradation of β-glucan in aqueous systems, via the Fenton reaction and ascorbic acid induced degradation of β-glucan, has been studied and is reviewed in this section. Nonetheless, the oxidative stability of β-glucan in multi-phased systems has not been investigated yet. Lipids are hydrophobic, but lipid oxidation can cause oxidation of other molecules in food through the interface. The co-oxidation of polymers during lipid oxidation and the role of polysaccharides in lipid oxidation are reviewed in this section. A schematic diagram showing the degradation and aggregation of polymers by various radical sources in food is presented in Figure 4.

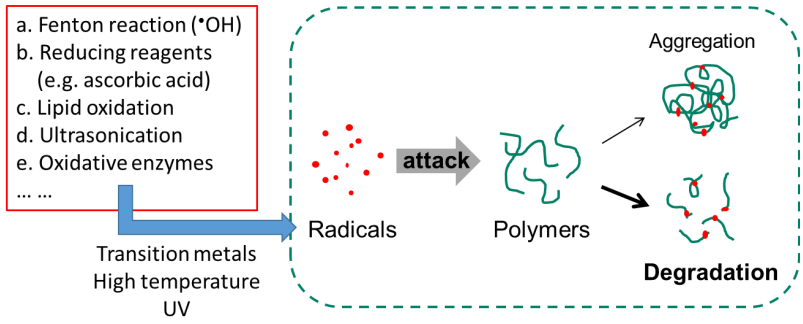

Figure 4. Radical sources in foods and the radical induced degradation and aggregation of polysaccharides.

2.2.1 Oxidative degradation of β-glucan in aqueous system

2.2.1.1 Fenton reaction induced degradation

Radicals can be formed through the Fenton reaction in the presence of hydrogen peroxide and transition metals such as iron (Reactions 1,2). Hydroxyl radicals (•OH) are the most reactive ROS which non-selectively attack a wide range of organic molecules. For carbohydrates, the hydroxyl radicals abstract the hydrogen from C-H moieties of the carbohydrate chain, leading to chain breakage or other modifications such as formation of carbonyls and carboxyls (von Sonntag, 1980; Potthast et al., 2006). Mäkinen et al. (2012) have found trace amounts of transition metals and hydrogen peroxide in β-glucan extracts from oat and barley, which can lead to radical formation and subsequent viscosity loss of the β-glucan extract. Mäkelä et al. (2015) showed a significant decrease in molecular weight of purified β-glucan with addition of 10 mM H_2O_2 or 70 mM and 1 mM iron(II) sulphate heptahydrate, and they later detected the formation of carbonyl groups and formic acid after oxidation (Mäkelä et al., 2017).

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \qquad (1)$$

$$H_2O_2 + Fe^{3+} \rightarrow Fe^{2+} + {}^{\bullet}OOH + H^+ \qquad (2)$$

The efficiency of the Fenton reaction is highly dependent on the pH of the solution, and acidic conditions are generally preferred (Jung et al., 2008). At an acidic pH, higher levels of iron can be dissolved in the aqueous solution which facilitates the hydroxyl radical production. At higher pH, the ferric ion will react with H_2O_2 (equation

2). If the pH is too high, iron will precipitate as $Fe(OH)_3$ and catalytically decompose the H_2O_2 to oxygen. The rate of Fenton reaction increases with increasing temperature. However, when the temperature increases to above 40-50°C, the efficiency of H_2O_2 utilization may decline due to an accelerated decomposition of H_2O_2 into oxygen and water.

2.2.1.2 Ascorbic acid accelerated degradation

Ascorbic acid is a common additive in foods such as cereal flours, beverages and dairy products to restore nutritional value loss during processing and boost the product's appearance, palatability and stability. Hydroxyl radicals can be formed in the presence of ascorbic acid and transition metals, since ascorbic acid is able to reduce transition metals (e.g. Fe(III) to Fe(II)) and simultaneously form H_2O_2 in the presence of molecular oxygen thus initiating Fenton-type reactions (Reactions 1, 3, 4) (Guo et al., 2002). In the reaction, ascorbate is oxidized to dehydroascorbate (DHA) while molecular oxygen is reduced to H_2O_2. The reduced Fe(II) and formed H_2O_2 then react to produce hydroxyl radicals (Reaction 1).

$$AA^- + 2Fe^{3+} \rightarrow DHA + 2H^+ + 2Fe^{2+} \quad (3)$$

$$AA^- + O_2 \rightarrow DHA + H_2O_2 \quad (4)$$

Molecular degradation of cereal β-glucan and extensive loss of viscosity in the presence of ascorbic acid (10 mM) and iron(II) sulphate have been shown(Kivelä et al., 2009). The extent of β-glucan degradation induced by ascorbic acid at room temperature was similar to the degradation extent when heating at 100ºC (Faure et al., 2013). Compared with ascorbic acid (10 mM), hydrogen peroxide (10 mM or 70 mM) induced β-glucan degradation and the reaction was faster in the presence of 1 mM iron(II) (Mäkelä et al., 2015).

2.2.2 Stability of β-glucan in multi-phased system

2.2.2.1 Lipid oxidation as a source of radicals

Lipids are one of the main components of food responsible for nutrition, taste and texture, and are essential in multi-phased food systems. Lipid oxidation is often a major determinant of food quality and shelf-life, especially when polyunsaturated fatty acids are favored for health promotion. Once started, the lipid oxidation reaction is self-propagating and self-accelerating (Schaich, 2005). During processing, lipid oxidation can be affected by factors such as transition metals, oxygen, light, temperature, enzymes, and antioxidants (Lingnert, 1992). A very small amount of pro-oxidants or antioxidants can cause large changes in oxidation rates.

Initiation	$LH \rightarrow L^{\bullet}$		(5)
Propagation	$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$		(6)
	$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet}$		(7)
	$LOOH + Fe^{2+} \rightarrow LO^{\bullet} + OH^{-} + Fe^{3+}$	(reducing metals)	(8)
	$LOOH + Fe^{3+} \rightarrow LOO^{\bullet} + H^{+} + Fe^{2+}$	(oxidizing metals)	(9)
	$LOOH \rightarrow LO^{\bullet} + {}^{\bullet}OH$	(heat and UV)	(10)
	$LO^{\bullet} + LH \rightarrow L^{\bullet} + LOH$		(11)
	$HO^{\bullet} + LH \rightarrow L^{\bullet} + HOH$		(12)
	$LO^{\bullet} + LOOH \rightarrow LOH + LOO^{\bullet}$		(13)
Termination	Radical recombinations and scissions		(14)
	$L^{\bullet} \quad L^{\bullet}$		
	$LO^{\bullet} + LO^{\bullet} \rightarrow$ Non-radical products		
	$LOO^{\bullet} \quad LOO^{\bullet}$ (aldehydes, ketones, ethers, alkanes, etc.)		

Scheme 1. Classical free radical chain reaction of lipid oxidation.

Lipid oxidation generates several kinds of radicals during the initiation and propagation reactions (Kamal-Eldin and Pokorný, 2005). The classical free radical chain reaction of lipid oxidation is shown in Scheme 1. Lipid oxidation requires an initiator or catalyst to form the initial lipid alkyl radicals ($L^{\bullet}$). Relatively unreactive $L^{\bullet}$ can react with oxygen to form reactive peroxyl radicals ($LOO^{\bullet}$) which abstract hydrogens from adjacent lipid molecules forming hydroperoxides (LOOH) and a new $L^{\bullet}$ (Reactions 6, 7). The radicals from lipid oxidation are able to abstract hydrogens from any molecules with labile hydrogens. The process continues indefinitely until no

easily abstractable hydrogen source is available or the chain reaction is interrupted (Reaction 14).

Notably, the lipid oxidation reactions accelerate when LOOHs accumulate and decompose to various radicals by metals, heat and ultraviolet (UV) light (Schaich, 2005). The metal reactions are heterolytic producing one radical and one ion (Reactions 8, 9), whereas heat and UV light induce homolytic scission forming two radicals which are alkoxyl radical (LO•) and hydroxyl radical (•OH) (Reaction 10). Both LO• and •OH can react much more rapidly and generally than LOO•, increasing the rate of the chain reaction. Production of radicals can be driven catalytically by trace levels of iron, copper and other redox-active metals in tissues (Schaich, 1992).

Apart from other cereal grains, oats contain high content of lipids ranging from 3% up to 18%, and the majority of the lipids (86-90%) are found in the endosperm (Banas et al., 2007). Thus, oat kernels or products have relatively elevated risk for lipid oxidation. Oat has a relatively high native lipolytic activity, especially lipase, which could rapidly release free fatty acids (Moltenberg et al., 1996; Yang et al., 2017). About 80% of the fatty acids in oats are unsaturated oleic, linoleic, and linolenic acids which can undergo different oxidation or isomerization reactions (Molteberg et al. 1996), and the oxidation of polyunsaturated fatty acids, especially linoleic and linolenic acids, can produce highly reactive radicals.

Cereals commonly have lipoxygenase and lipoperoxidase activities which are relatively high in barley and wheat compared with rye and oats (Lehtinen, 2003). With even small amounts of these enzymes, LOOH decomposes to reactive radicals which may attack other molecules including β-glucan. The typical heat processes for oats, including steam stabilization and kilning, contribute to the flavor development and inactivation of most enzymes. Additionally it should be noted that overprocessing of oats may cause oxidative rancidity and bitter flavors (Molteberg et al., 1995). Study have shown that the hexanal concentration rose rapidly after heat treatment of oats (e.g. after kilning and drying) (Klensporf and Jelen, 2008). In addition, lipoxygenase is utilized in the bleaching of flour and improvement of rheological properties of dough (Hoseney et at. 1980; Bahal et al. 2013). The effect of oxidative enzymes and overprocessing on lipid oxidation and its co-oxidation of other food components has not been much studied.

2.2.2.2 Co-oxidation of other molecules during lipid oxidation

Lipids are dispersed in a heterogeneous matrix of starch, proteins and fiber. Co-oxidation of non-lipid molecules such as protein and carbohydrate is one of the mechanisms in the termination step of lipid oxidation (Schaich, 2005; 2013). As a result, cross-linking, degradation and other changes in chemical and physical properties of these macromolecules have been shown. For example, oxidized lipids can react with proteins present in food and produce polymeric protein complex causing poor protein digestibility (Obando et al., 2015). Kawakishi et al. (1983) showed oxidative degradation of β-cyclodextrin induced by autoxidation of linoleate in a solid system, which was enhanced by increased moisture content in the system. Changes in molecular weight and crystalline structure of corn starch by coexisting methyl linoleate has been shown in solid systems as well (Komiya et al., 1990). Ishii et al. (1976) have found changes in physico-chemical properties e.g. gelatinization temperature, rheological properties and reducing sugar content of starch when dipped in oxidized linseed oil at 40°C.

As reviewed, the studies on co-oxidation of polysaccharides with lipids were scarce and most of them were done in systems with low moisture content. Nelson and Labuza (1992) have stated that at both very high and very low water activities, lipid oxidation rates are high compared to the lipid oxidation rate at intermediate water activities. In many instances, food processing protocols or products are in liquid emulsion systems, which could increase the risk of lipid oxidation and co-oxidation of polysaccharides due to the increase in moisture content and mobility of pro-oxidants (McClements and Decker, 2000), and this requires more studies.

2.2.2.3 Role of polysaccharides in emulsion stability

Emulsion is a typical multi-phased system and it is thermodynamically unstable due to the energy needed to increase the surface area between the two immiscible phases. Therefore, emulsifiers are used as surface active molecules to reduce the tension of the surface and form a protective membrane against aggregation and coalescence (McClements, 2005). Polysaccharides can improve the physical stability of emulsions by adsorbing to the oil droplet surfaces and increasing the steric and electrostatic repulsion between the droplets, or by thickening and gelling the aqueous phase (Dickinson, 2003; Guzey and McClements, 2006). Polysaccharides containing functional side chains such as negative charge (gum arabic, modified starches, modified celluloses, certain pectins and galactomannan, etc.) and

polysaccharide-protein conjugates have been extensively studied to stabilize emulsions in the past years (Dickinson 1998; Harnsilawat et al., 2006). For example, the charged groups in polysaccharide can have ionic or non-ionic interactions with proteins and affects the emulsion stability, depending on the aqueous environmental conditions such as solvent, pH, Ca^{2+} content.

Cereal β-glucan, as a neutral polysaccharide, can stabilize an emulsion mainly by increasing the viscosity of the continuous phase, especially in the case of high molecular weight β-glucan (Burkus and Temelli, 2000). β-Glucan with low molecular weight (40 000 g/mol) has been shown to stabilize an emulsion by network formation in the continuous phase (Kontogiorgos et al., 2004). β-Glucan extracts may exhibit mild surface activity due to the presence of impurities such as proteins, but β-glucan (1%) alone without using other emulsifiers cannot stabilize an emulsion (Kontogiorgos et al., 2004). To study the mechanism of action of polysaccharides in emulsions, surfactants such as Tween 20 which do not affect the continuous phase viscosity nor the interactions between the droplets are often used for the stabilization of the emulsion (Kontogiorgos et al., 2004).

Compared to oil in bulk form, lipids are known to be oxidized much more readily in emulsions due to the large surface area of the emulsion which facilitates interactions between lipids and water soluble pro-oxidants such as iron (McClements and Decker, 2000). Oxygen dissolved in the aqueous phase has more potential to access the oil phase when the interfacial area is high in emulsions. Lipid hydroperoxides are more polar due to the presence of oxygen, which causes them to diffuse toward the water-oil interface of emulsions (Nuchi et al., 2002). Transition metals dissolved in the water phase decompose the LOOH and produce free radicals (Figure 5). Free radicals generated at the oil-water interface may diffuse into droplets and promote lipid oxidation, or travel to the water phase causing oxidation of the water soluble components (McClements and Decker, 2017). Oil-water interfaces in food emulsions have a major impact on the lipid oxidation pathways by affecting the location and reactivity of pro-oxidative transition metals, lipid hydroperoxides, minor lipid components, free radical scavengers and metal chelators (Waraho et al., 2011). In addition, the emulsification process may introduce heat, shear forces and radicals which enhance oxidation, as reviewed by Berton-Carabin et al. (2014).

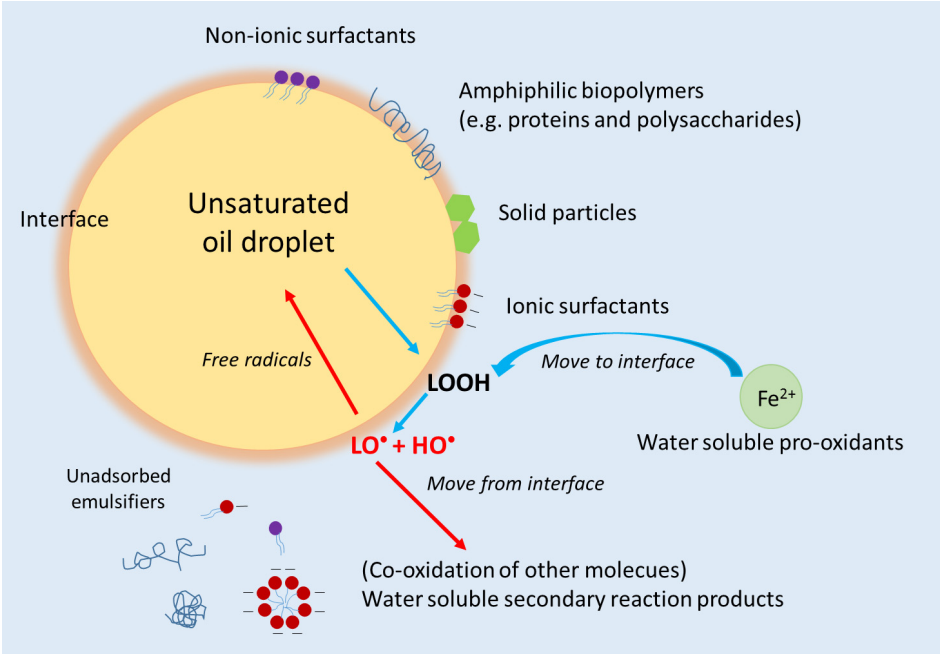

Figure 5. Schematic representation of possible components of an oil-in-water emulsion and the role of interface in the lipid oxidation process. LOOH is lipid hydroperoxide and LO• is lipid alkoxyl radicals.

In addition to the improvement of phase stability, some polysaccharides have been shown to retard the lipid oxidation process in emulsions. The mechanisms of lipid oxidation retardation by polysaccharides include free radical scavenging, viscosity enhancement, and transition metal binding by the polysaccharides (Matsumura et al., 2003; Shimada et al., 1992, 1996). Matsumura et al. (2003) showed that gum arabic and soluble soybean polysaccharides (2.5 or 5% w/w), which have covalently-bound peptide moieties, inhibited lipid oxidation in emulsions, whereas maltodextrin and pullulan did not show an inhibitory effect. They found that the soluble soybean polysaccharides retarded lipid oxidation mainly by radical scavenging rather than viscosity or transition metal chelation. Another study showed that the antioxidant effect of xanthan gum in an oil-in-water emulsion was due to the chelation of the transition metal and the viscosity increase in the continuous phase which reduced the oxygen diffusion rate and the oil droplet collision probability (Shimada et al., 1996). However, little has been done to investigate the change in the polysaccharide due to lipid oxidation. It has been shown that the interfacial or unabsorbed emulsifier in emulsion system can be modified due to lipid oxidation (Berton et al., 2012). As

indicated in Figure 5, the radicals produced in lipid oxidation may move to the aqueous phase and cause oxidation of components including water soluble polysaccharides, in the aqueous phase.

2.2.2.4 Associated compounds in plant polysaccharides

The impurity and composition of the studied polysaccharides are rarely considered when studying the bioactivity of polysaccharide extracts. Polysaccharides from various sources may contain associated bioactive compounds or groups that contribute to the antioxidant activity as well as other properties of polysaccharides. In cereals, ferulic and coumaric acids are the main bound phenolic acids and they were predominantly (~90%) bound to water-unextractable non-starch polysaccharide (Rao and Muralikrishna, 2004). Studies have shown that the ferulate complexed with arabinoxylans contributed to the antioxidant activity of arabinoxylans (Malunga and Beta, 2015). The phenolic compounds are often extracted by organic solvents such as methanol, ethanol, acetone or the aqueous phase of solvent mixtures. Differently, more than 90% of phytic acid has been found in the water-soluble fiber fraction of oats **(**Frølich and Nyman, 1988) and the soluble fiber fraction in oats was mostly β-glucan. Kivelä (2011) has reported that the phytate content in the oat β-glucan extracts can be up to 6% (dm basis). Ghotra et al. (2007) have shown that the isolated oat and barley β-glucan contained total phosphorus at 0.920 and 0.170%, w/w, respectively. Further purification could not remove all of the phosphorus with 0.201% and 0.092% (w/w) phosphorus remaining in the purified oat and barley β-glucans, respectively. It has been shown that associated phytate in dietary fiber largely contributed to the inhibition of mineral absorption of the dietary fiber (Persson et al., 1991). Kennefick and Cashman (2000) have found the inhibitory effect of wheat fiber extract on calcium absorption in caco-2 cells was mainly from the associated phytate rather than fiber per se. Phytate is very likely a co-passenger of cereal β-glucan, and whether it affects the antioxidant activity and physico-chemical properties of β-glucan warrants more studies.

2.3 Introduction to phytic acid

2.3.1 Phytic acid distribution and content in cereals

Phytate, namely phytin as well, is the salt of phytic acid (*myo*-inositol-1,2,3,4,5,6-hexakis dihydrogen phosphate) and it originates from plants. It serves as a storage form of phosphorous and minerals and it accounts for 69-90% of total phosphorous content in cereals, legumes, nuts and oil seeds (Lott et al., 2000). Phytic acid has a significant role in plant metabolism, especially during germination of the seeds. Phosphate along with minerals such as calcium and magnesium becomes available when the phytate is hydrolyzed during germination and development of the seedling. Another physiological function of phytic acid is to control the metabolism by binding the multivalent cations required for cellular process (Cabrera-Orozco et al., 2013).

The main sources of phytate in the daily diet are cereals and legumes; the leafy vegetables and fruits do not contain phytic acid. In cereals, phytate is located in the aleurone layer (e.g. wheat, barley, oat and rice) and the germ (e.g. maize) while the endosperm is almost free of phytate (O'Dell et al., 1972). In oats and barley, phytate is in protein storage vacuoles as globoids in the aleurone cell layer (Figure 6, stained red) and it makes 0.42-1.16% and 0.38-1.16% of the whole grain on a dry matter basis, respectively (Table 1). Saastamoinen et al. (1992) reported that the total phytic acid content varied between 0.56% and 0.87% in oats cultivated in Finland. In general, the content of phytate varies from 0.14 to 2.22% in cereals, 0.08 to 5.46% in cereal fractions, and 0.03 to 2.41% in cereal-based foods (Wise, 1983). Strong similarities were observed in the distribution of Fe and Zn and P in the bran tissue, indicating that Fe and Zn are confined to the spherical phytate globoids in the aleurone (Singh et al., 2013). The study also suggested that, on intact whole grains, about 80% of the Fe is present in ferric form, and the remainder is in ferrous form. The phytic acid is associated with brans, therefore mineral, phosphorous and phytate content is much higher in the bran fraction than in the whole grain (Steiner et al. 2007). The phytate content of different fractions of oat, barley and wheat is collected in Table 1.

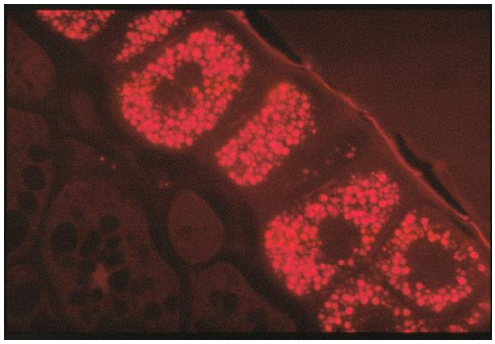

Figure 6. Microscopy of phytate in aleurone layer stained with Acriflavine (left, red) from slides of oats embedded in GMA resin (sectioned at about 4 µm thick. (Courtesy by Shea Miller, Agriculture and Agri-Food Canada)

Table 1. Phytate content (dry matter) of grain, flour and bran in oat, barley and wheat.

	Phytate content, % (dm)		
	Grain	Flour	Bran
Oat	0.42-1.16	0.4-0.74	0.6-2.41
Barley	0.38-1.16	0.63	
Wheat	0.39-1.35	0.25-1.37	2.02-5.84

(Data are integrated from Reddy and Sathe, 2002; Garcia-Estepa et al., 1999; Lolas et al., 1976)

2.3.2 Phytic acid interaction with mineral and protein

Phytate is negatively charged over wide range of pH (pH 1-13). It strongly chelates positively charged cations such as iron, zinc, magnesium and calcium, and interacts with starch and proteins (Figure 5). Phytate has been regarded as an antinutrient because it impairs the bioavailability of some essential minerals during gastro-intestinal passage. The interaction between phytic acid and minerals and the solubility of these complexes are affected by factors including pH, the specific mineral, mineral and phytate concentration and ratio (Bohn et al., 2008). The complex of phytic acid and minerals can be soluble in acidic stomach conditions but may precipitate in the small intestine where the pH is higher, resulting in poor absorption of minerals and trace elements (Schlemmer et al., 2009). Animal studies have shown that the intestinal fermentation lowers the inhibitory effect of phytate on mineral utilization in rats (Lopez et al., 1998; Lopez et al., 2002). Lopez et al. (2002) showed that resistant starch which is a fermentable dietary fiber produced short chain fatty acids which lowered the pH and formed absorbable complexes with ions, and therefore counteracted the inhibitory effect of phytate in the large intestine.

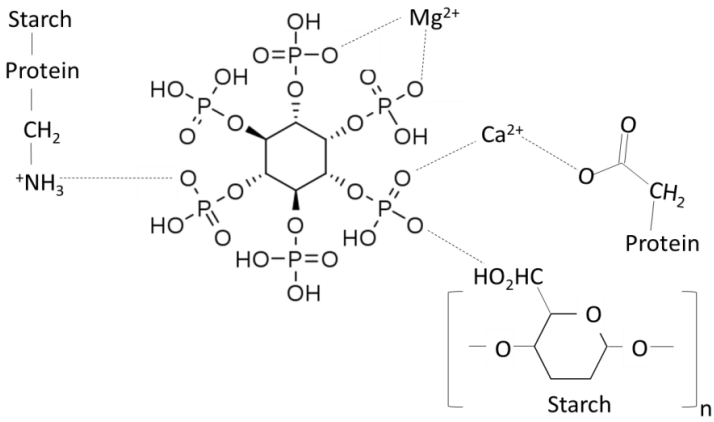

Figure 7. Phytic acid interactions with minerals, proteins and starch (Modified from Thompson 1987)

When the mineral:phytic acid molar ratio is ≥ 1, the calcium and magnesium phytates initially precipitate in the pH 4-6 and 5-7 ranges, respectively. At lower mineral:phytic acid molar ratios, calcium and magnesium phytates are soluble throughout the pH range. In most diets, minerals such as iron, zinc and copper are present only in small

quantities which alone are insufficient to precipitate phytic acid (Wise and Gilburt, 1983). However, if sufficient calcium or magnesium is present, these trace minerals will co-precipitate with calcium/magnesium phytates. Moreover, only inositol hexaphosphate (IP6) and inositol pentaphosphate (IP5) have a negative effect on the bioavailability of minerals, and the other hydrolytic products have a poor capacity to bind minerals, or the complexes formed are more soluble (Sandberg et al., 1999). Food processing degrades phytic acid to lower *myo*-inositol phosphates esters, which do not have a high capacity to chelate minerals.

Phytic acid is reactive with proteins, forming insoluble complexes and therefore affects the functionality of the proteins. At low pH and low cation concentrations, phytic acid-protein complexes are formed due to direct electrostatic interactions, while at pH > 6 to 7, a ternary phytic acid-mineral-protein complex is formed (Cheryan and Rackis, 1980) (Figure 5). These complexes may affect the protein structure, decreasing the enzyme activity, function, solubility, absorption and protein digestibility (Thompson, 1987). Phytic acid may inhibit proteolytic (pepsin, trypsin, chymotrypsin), amylolytic (amylase) and lipolytic (lipase) enzymes due to the nonspecific nature of inositol phosphate-protein interactions and the chelation of calcium ions which are essential for the activity of trypsin and α-amylase (Cheryan and Rackis, 1980; Thompson et al., 1987; Coulibaly et al., 2011).

2.3.3 Phytic acid reduction

Much effort in breeding and processing has been made towards reduction of phytate in foods to minimize the mineral malnutrition. Milling of cereals removes phytate but also removes the majority of the minerals and dietary fibers (Bohn et al., 2008), therefore it is not a nutritional solution. Phytate is heat stable up to 100°C, therefore it is not easily degraded by conventional heat processing such as cooking, autoclaving or roasting (Sathe and Venkatachalam, 2002; Schlemmer et al., 2009). It can be reduced by acid hydrolysis, or endogenous or exogenous phytase, which belongs to the group of esterases.

Phytase is a phosphatase occurring naturally in cereals. It can hydrolyse phytate to *myo*-inositol and inorganic phosphate along with production of intermediate *myo*-inositol phosphates (e.g. IP5, IP4, and IP3). Phytase is inactive in dry cereals while it becomes active when the moisture content increases. In cereal processing such as malting, fermenting and soaking, phytase can hydrolyze the IP6 in a stepwise

process. Optimal conditions for wheat phytase are 55°C and pH 5 (Türk et al., 1996). Barley has a relatively high phytase activity in contrast to oats. Oats have low phytase activity prior to processing, and the phytase activity is even further reduced after thermal treatment (e.g. kilning), which is routinely performed on commercial oat products (Bartnik and Szafranska, 1987; Larsson and Sandberg, 1992). Therefore, phytate content remains high in oat bran fractions or whole grain products from oats. Fredlund et al. (1997) reported that due to the low phytase activity in oats, phytate reduction for oats by incubation for 24h at optimal temperature with either water or acetate buffer (pH 4.8) was only 8-26% while the reduction for wheat, rye and barley was up to 99%. Barley was found to have the fastest phytate decomposition among the four cereals with almost complete degradation after the first hour of incubation at 55°C (Larsson and Sandberg, 1992). Extensive phytate degradation in oats occurred only after long-term germination, which caused an extensive breakdown of soluble fiber, especially β-glucan (Hubner et al., 2010). It seems that reduction of phytate without degrading β-glucan in the material is challenging.

2.3.4 Antioxidant effect of phytate

Phytic acid is a natural plant antioxidant which suppresses iron-catalyzed oxidative reactions by forming a unique iron chelate. Phytic acid maintains iron in the Fe(III) oxidation state and obstructs generation of hydroxyl radicals and other ROS by occupying all of the available iron coordination sites (Graf et al., 1984; Graf et al., 1987). In plants, it controls the generation of cellular free radicals which are extremely toxic, and serves as a potent antioxidant in the preservation of seeds (Graf and Eaton 1990). Oats contain a high content of lipids and the presence of phytate in oats can be a naturally defense system against oxidation. Adding phytic acid to foods can inhibit the Fenton reaction, lipid peroxidation and concomitant oxidative spoilage. In a study by Sakač et al. (2010), phytic acid did not inhibit the thermal oxidation (60°C, 24h) of soybean oil, but inhibited the catalytic oxidation (Fe(II), 23°C, 3h) of soybean oil, which confirmed the mechanism of antioxidant activity was the chelation of Fe^{2+} by phytic acid. Colonic cancer has been associated with dietary fat intake and it can be reduced by many fiber-rich foods which may contain high levels of phytate (Coulibaly et al., 2011). Phytic acid reduces the formation of radicals which randomly attack most of the biomolecules, and therefore suppresses oxidation reactions including lipid peroxidation and DNA damage (Graf and Eaton, 1993).

In addition to its antioxidant and anticancer activity, a positive role of phytic acid in controlling the levels of blood glucose and cholesterol, inhibition of calcium salt crystallization and prevention of renal stone formation have been reported (Review by Schlemmer et al., 2009). Interestingly, the management of blood glucose and cholesterol levels has also been found with cereal β-glucan. The co-existence of phytate and cereal β-glucan opens a thought that phytate may contribute to the physico-chemical properties and physiological functionalities of cereal β-glucan.

In summary, the oxidative degradation of cereal β-glucan in aqueous systems and the role of polysaccharides in the physical and chemical stability of emulsions were reviewed. Furthermore, lipid oxidation and co-oxidation of other molecules such as protein and starch were introduced. The review however highlights the need to study the oxidative stability of β-glucan in the presence of oxidized lipids and the role of β-glucan in retardation of lipid oxidation. Polysaccharide extracts can carry various bioactive compounds. As reviewed, these associated compounds can contribute to the mineral binding and the antioxidant activity of the polysaccharides. Phytic acid, as a co-passenger of cereal β-glucan in the extraction process, has been noticed earlier and whether it plays a role in the oxidative stability of β-glucan needs to be studied.

3 AIMS OF THE STUDY

The aim of the study was to understand the oxidative stability of cereal β-glucan in multi-phased systems. Radicals from lipid oxidation were hypothesized to attack cereal β-glucan causing its oxidative degradation. An oil-in-water emulsion model system which facilitated the dispersion of hydrophobic lipids in the aqueous β-glucan solution was utilized to evaluate this hypothesis. Whether the β-glucan simultaneously retards the lipid oxidation was also investigated. The retardation mechanisms were considered to either involve β-glucan and/or phytate which is a co-passenger in β-glucan. Furthermore, the phytate as a well-known antioxidant was considered to protect the β-glucan from oxidation.

The specific objectives were to:

i) Evaluate the degradation of cereal β-glucans induced by lipid oxidation (Study I, II);

ii) Investigate the retardation of lipid oxidation by cereal β-glucans, and determine the role of β-glucan molecular weight, source and residual phytate in the retardation (Study I, II);

iii) Examine the role of phytate in the oxidative stability of cereal β-glucan (Study I, II, III).

4 MATERIALS AND METHODS

4.1 Materials and sample preparation

Methyl linoleate (ML, Nu Check Prep. Inc., Elysian, MN, USA, purity > 99%) and purified rapeseed oil (RO) were used for lipid oxidation (Study I, II). ML and RO were pre-oxidized at 55ºC in an open container to produce lipid hydroperoxides (LOOH). Oils with different extent of pre-oxidation including mildly oxidized ML (MML, peroxide value (PV) = 740 mequiv/kg oil), highly oxidized ML (HML, PV = 1630 mequiv/kg oil), non-oxidized RO (NRO, PV = 5 mequiv/kg oil), oxidized RO (RO, PV = 334 mequiv/kg oil) and highly oxidized RO (HRO, PV= 782 mequiv/kg oil) were produced (Study I). Oil-in-water emulsions (stock emulsion) were prepared using Tween 20 (polyoxyethylene sorbitan monolaurate, Merck-Schuchardt, Germany) as an emulsifier and were emulsified by an ultrasonicator (Study I, II).

Commercial barley β-glucan with high molecular weight (495 000 g/mol, BBG-H) and low molecular weight (245 000 g/mol, BBG-L), oat β-glucan with high molecular weight (361 000 g/mol, OBG-H) and low molecular weight (272 000 g/mol, OBG-L) were purchased from Megazyme, Ireland (purity > 94%, dw basis). The specific β-glucan samples used in Study I-III are presented in Table 2 and Figure 8. β-Glucan solutions were prepared by first wetting with 99.5% ethanol and then mixing at 85ºC for 2 hours after adding water.

The residual phytate in the β-glucan samples was removed by an ion-exchange resin (Amberlite IRA-410 Chloride form 20-25 mesh, Sigma-Aldrich, Saint Louis, USA) (Study II, III). The resin and β-glucan solution were mixed at 4ºC for 2 hours and then filtrated. The filtrate was dialyzed against MilliQ water, precipitated with ethanol and freeze-dried. In the study of the kinetic of β-glucan degradation induced by the Fenton reaction (Study III), the β-glucan solution obtained after phytate removal procedure was concentrated by evaporation in order to maintain the stability of β-glucan. Phytic acid (50% solution, ICN Biomedicals Inc., USA) and L(+)-ascorbic acid (J.T. Baker, Avantor Performance Material, USA) were used in Study III.

4.2 Oxidation conditions

To study the co-oxidation of β-glucan with oxidized lipids, the stock emulsions and β-glucan solution were mixed and vortexed. The oxidation was accelerated by adding iron (II) sulphate heptahydrate ($FeSO_4 \cdot 7\ H_2O$, Merck, Germany). The final emulsion (BBG-H + oil + Fe) contained 0.56% (w/v) β-glucan, 1.0% oil, 0.1% emulsifier and 1.0 or 0.1 mM Fe(II) (Study I). In Study II, a smaller amount of Fe(II) (0.1 mM) was used to create milder oxidation conditions in order to observe the initial stages of lipid oxidation during the measurement. Four β-glucan samples with and without phytate were added into the stock emulsion (Study II). All emulsion samples were stored at room temperature or at 40ºC for 7 days with vortexing once per day. A summary of the oxidation conditions and the final concentrations of the components in the mixture used in Study I-III is shown in Table 2.

Fenton reagents including Fe(II) stock solution and hydrogen peroxide (H_2O_2, 30%, Merck, Germany) were separately added into the β-glucan stock solution to induce oxidative degradation in Study III. Various iron concentrations (0.1 mM, 0.5 mM or 1 mM) and H_2O_2 concentrations (10 mM or 1 mM) were used to induce different degrees of β-glucan oxidation. To obtain similar initial viscosity (170 mPa. s at shear rate 10^{-s}), different concentrations of oat β-glucan (OBG-H) and barley β-glucan (BBG-H) were used, which were 1.2% and 0.8% in the final solution, respectively. The oxidation was carried out at room temperature and was measured for 7 days.

To further investigate the role of phytic acid in the stability of β-glucan, commercial phytic acid was added into the phytate removed β-glucan solution or BBG-H solution which originally contained a negligible amount of residual phytate (Study III). The concentration of phytic acid in the final β-glucan solution was either 0.2 mM, 0.5 mM, or 2.5 mM. To study the competition of ascorbic acid and phytic acid in the Fenton reaction, L(+)-ascorbic acid (0.5 mM or 0.1 mM) was added into the BBG solution before adding Fenton reagents. The evaluated factors and the samples/treatments included in Study I-III are provided in Figure 8.

Table 2. Summary of the β-glucan source, concentration, phytate content and reagents used for oxidation in Study I, II and III. The concentration of β-glucan and Fe(II) is the final concentration in the reaction mixtures.

	β-Glucan source	β-Glucan concentration % (w/v)	Phytic acid concentration % dm basis	Oxidation source	Fe(II) concentration mM
Study I	BBG-H	0.56	0.04	RO or ML	1.0
Study II	BBG-H	0.56	0.04	ML	0.1
	BBG-L	0.56	0.24	ML	0.1
	OBG-H	0.56	1.23	ML	0.1
	OBG-L	0.56	0.82	ML	0.1
Study III	BBG-H	0.8	0.04	H_2O_2	0.1 or 0.5
	OBG-H	1.2	1.23	H_2O_2	0.1, 0.5 or 1.0

Note: BBG-H is barley β-glucan with high M_w, BBG-L is barley β-glucan with low M_w, OBG-H is oat β-glucan with high M_w, and OBG-L is oat β-glucan with low M_w. RO is rapeseed oil, ML is methyl linoleate. The concentration of RO or ML was 1% (w/w) and the concentration of H_2O_2 was 10 mM.

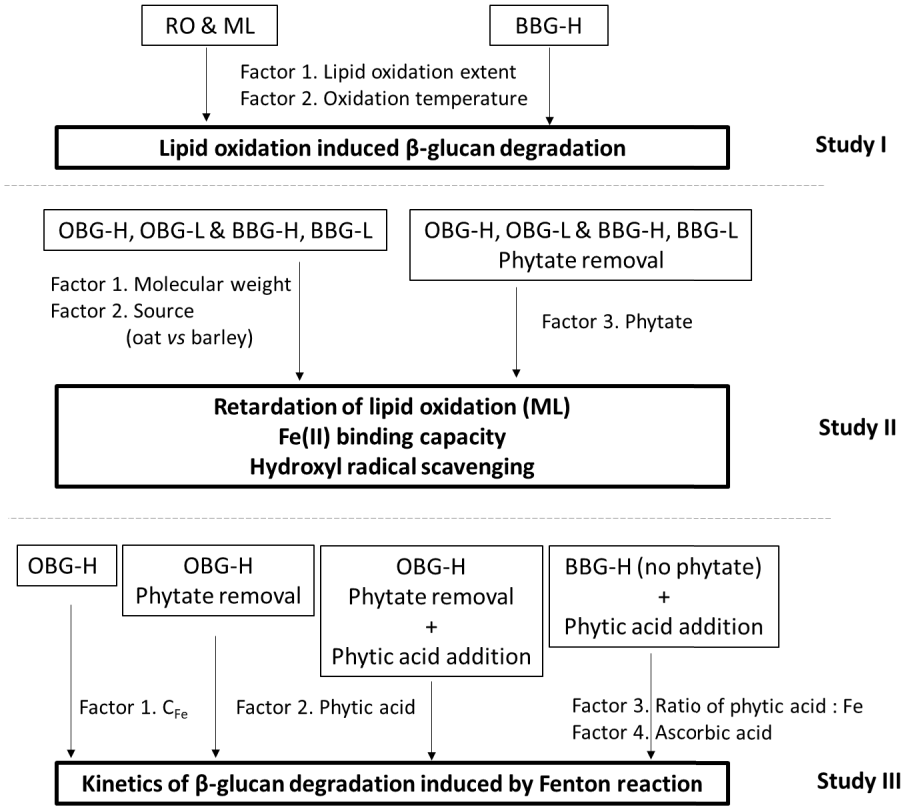

Figure 8. The outline of the experimental design in studies I-III. RO is rapeseed oil and ML is methyl linoleate. BBG-H is barley β-glucan with high M_w; BBG-L is barley β-glucan with low M_w; OBG-H is oat β-glucan with high M_w; and OBG-L is oat β-glucan with low M_w. C_{Fe} is the concentration of Fe(II).

4.3 Measurements

4.3.1 Analysis of mineral and phytate content (Study II)

Mineral content (P, Ca, Mg, K, Fe, Mn, Zn and Cu) of the β-glucan samples was determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo Scientific iCAP 6000). The phytic acid content of the samples before and after phytate removal was measured as phosphorus released by phytase and alkaline phosphatase using the phytic acid/total phosphorus assay from Megazyme Ireland. The mineral and phytate content was calculated based on the fresh weight of the β-glucan sample.

4.3.2 Analysis of lipid oxidation (Study I and II)

The peroxide value of the emulsion samples (0.56% β-glucan, w/v) was measured with the ferric thiocyanate method as described by Ueda et al. (1986). Ethanol was added to precipitate the β-glucan in the sample and the precipitate was removed by filtration. Hexanal produced during the oxidation of the emulsion was measured with headspace gas chromatography (GC, Perkin Elmer, USA) using 0.5 g (0.49–0.51 g) of the emulsion samples.

4.3.3 Analysis of antioxidant activity (Study II)

The iron binding capacity of different β-glucan samples (0.6% β-glucan, w/v) was determined according to Dinis et al. (1994). Ferrozine (3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4''-disulfonic acid sodium salt, Sigma-Aldrich, USA) forms a magenta colored solution with ferrous ions. The iron binding capacity was calculated as:

$$\text{Iron binding capacity} = (1 - A_p/A_0) \times 100\ \% \qquad \text{(Equation 1)}$$

where A_p and A_0 are absorbance of β-glucan solution and blank, respectively.

The measurement of hydroxyl radical scavenging ability of β-glucan samples (0.6% β-glucan, w/v) was based on the competitive scavenging of ·OH by salicylate and the polysaccharide as described by Smirnoff and Cumbes (1989). The hydroxylation of salicylate by ·OH is accompanied by a color change that is measured spectrophotometrically. The hydroxyl radical scavenging ability was calculated as:

·OH scavenging ability % = [1 – (A_1 – A_2) / A_0] x 100 % (Equation 2)

where A_0 was the absorbance of the control without β-glucan, A_1 was the absorbance in the presence of β-glucan, and A_2 was the absorbance without sodium salicylate.

4.3.4 Analysis of β-glucan degradation (Study I, II and III)

β-Glucan degradation was determined by measuring changes in viscosity and M_w during oxidation. Viscosity measurement was carried-out with a Haake RheoStress 600 rheometer (Thermo Electron GmbH, Germany) using a cone and plate geometry (35 mm, 2°). The measurement was done at 20°C with a stepwise rotational program (shear rate increases logarithmically from 10 s^{-1} to 100 s^{-1}) or at a constant shear rate (10 s^{-1}). The apparent viscosity at shear rate of 10 s-1 was used to compare the effects of different oxidation conditions. The measurement was carried out in triplicate.

The M_w of β-glucan was analysed in 0.01 M LiBr/DMSO (dimethyl sulfoxide) solution using high pressure size exclusion chromatography (HPSEC) as described by Mäkelä et al. (2015). The oxidized samples were stored at -20°C until they were analysed. Prior to HPSEC analysis, the frozen samples were melted, diluted directly to 2 mg/ml with 0.01 M LiBr/DMSO, heated at 85°C for 30 min and then stored at room temperature overnight to ensure proper dissolution. Non-oxidized β-glucans were analysed as a control. The molecular weight measurement for each sample was measured in duplicate.

4.3.5 Modelling of the degradation kinetics (Study III)

The oxidative degradation of β-glucan was evaluated by viscosity reduction. The kinetic of β-glucan oxidative degradation was modelled using zero-order, first-order, and second-order models with the following equations:

$V_t - V_0 = kt$ (Equation 3)

$Ln(V_t/V_0) = kt$ (Equation 4)

$1/V_t - 1/V_0 = kt$ (Equation 5)

where k is the rate constant of viscosity decrease during oxidation; t is the oxidation time (min); V_t and V_0 (mPa·s) are the viscosity at oxidation time t and time 0, respectively. The best fit reaction order was obtained through a trial-and-error procedure. The model with the best correlation coefficient (R^2) was used to indicate the β-glucan oxidative degradation behavior.

4.4 Statistical analysis

Statistical analyses were performed on Statistical Package for the Social Science (SPSS Statistics version 23, IBM), using one-way analysis of variance (ANOVA) followed by post hoc Turkey test. Differences were considered as significant at $p < 0.05$. All the results were presented as means ± standard deviation.

5 RESULTS

5.1 Degradation of β-glucan induced by lipid oxidation (I, II)

The Study I showed that there was a significant degradation of β-glucan in the presence of oxidized lipids in the oil-in-water emulsion model system. The extent of β-glucan degradation was dependent on the initial lipid oxidation status (peroxide value), iron concentration, oxidation temperature, as well as the oil type. The peroxide value and hexanal formation increased during the oxidation process which indicated the continuous production of lipid radicals. The molecular weight decrease of β-glucan corresponded well with the viscosity drop when oxidizing with the same type of oil.

The degradation of β-glucan was more severe when higher temperature and higher concentrations of lipid hydroperoxides were used. When oxidized at 40ºC, the viscosity decrease was almost 3-fold compared to oxidation at room temperature (Figure 9). Aggregates which precipitated to the bottom of the reaction bottle were observed in this most oxidized sample. Control samples without addition of Fe(II) (BBG-H + OIL) also had a significant viscosity drop (10%) at elevated temperature. No significant viscosity change was observed in control samples which did not contain oil (BBG-H and BBG-H + Fe). Highly oxidized methyl linoleate (HML) caused more decrease in viscosity than mildly oxidized methyl linoleate (MML) at room temperature. Similarly, highly oxidized rapeseed oil (HRO) caused more degradation of β-glucan than oxidized rapeseed oil (RO) at 40ºC. The oil droplets were smaller in ML stock emulsion compared to RO stock emulsion.

The addition of Fe(II) accelerated the lipid oxidation and the degradation of β-glucan. When 1 mM Fe(II) was added, lipid oxidation induced s significant viscosity decrease (around 20%) in the BBG-H solution after oxidation at room temperature for 7 days (Figure 9). However, the lipid oxidation induced less of a viscosity decrease (8%) when a lower amount of Fe(II) (0.1 Mm) was used (Study II).

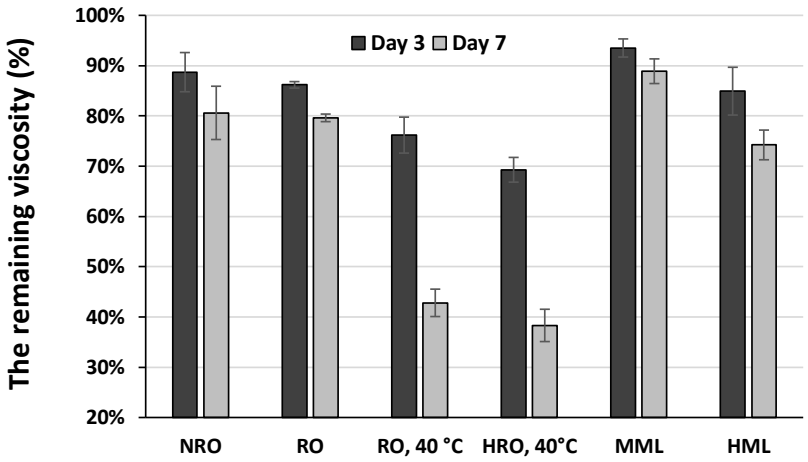

Figure 9. The remaining viscosity of β-glucan in emulsions (0.56% BBG-H + 1% oil + 1 mM Fe(II)) after oxidation for 3 and 7 days. NRO is non-oxidized rapeseed oil, RO is oxidized rapeseed oil, HRO is highly oxidized rapeseed oil; MML and HML are mildly and highly oxidized methyl linoleate, respectively. Error bars show standard deviation.

5.2 Retardation of lipid oxidation by β-glucan samples (I, II)

5.2.1 Role of β-glucan molecular weight and source in retardation of lipid oxidation

In study I, it was found that the hexanal amount was reduced in the emulsions containing β-glucan compared to the control emulsion which did not contain β-glucan. In study II, the retardation of lipid oxidation by β-glucan samples was seen clearly. The emulsions containing β-glucan produced around 50% less lipid hydroperoxides and hexanal compared to that in the control emulsion, except for BBG-H which produced more hexanal than the control emulsion.

β-Glucans with lower M_w (weight-average molecular weight) had better retardation of lipid oxidation than β-glucans with high M_w (Table 3). Furthermore, oat β-glucans decreased the peroxide value and hexanal content more efficiently compared to barley β-glucans when the β-glucans with similar M_w were compared (OBG-L > BBG-L, OBG-H > BBG-H). It therefore initially seemed that the differences between oat and barley β-glucans on retardation of lipid oxidation were attributed to the source

and molecular weight of β-glucan. In addition, it was observed that the emulsions with incorporation of low M_w β-glucan showed better phase stability compared to the emulsions with high M_w β-glucan.

5.2.2 Mineral and phytic acid contents of purified oat and barley β-glucans

The mineral contents varied in all β-glucan samples although they were highly purified (purity> 94%) (Study II, Table 1). Oat β-glucans contained significantly higher amounts of phosphorous (P) as well as Ca, Mg, Fe, Mn and Zn than barley β-glucans. Among the four β-glucan samples, OBG-H contained the highest amount of P (3.9 x 10^3 µg/g) and other minerals, and BBG-H contained the lowest amount of P (0.14 x 10^3 µg/g) as well as other minerals. The phytic acid content of fresh matter in OBG-H, OBG-L, BBG-L and BBG-H was 1.23%, 0.82%, 0.24% and 0.04%, respectively. Most of the P in the β-glucan samples was from phytic acid since the content of P released from phytic acid was similar to the total P content. Phytate removal by ion exchange reduced the phytic acid content in all β-glucan samples to lower than 0.05 % of fresh matter.

5.2.3 Role of residual phytate in retardation of lipid oxidation

In general, β-glucan samples with a high content of residual phytate showed significant retardation of lipid oxidation. BBG-H which contained low content of residual phytate decreased the peroxide value but did not retard the hexanal production in the emulsion (Table 3). When the residual phytate was removed from β-glucan samples by ion exchange, none of the β-glucan samples could retard the lipid oxidation. In this case there was no difference among the oat and barley β-glucan samples with different molecular weight (Table 3; Study II, Figure2). The peroxide value and hexanal content in all β-glucan emulsions were similar to the control emulsion.

To investigate the mechanisms of the lipid oxidation retardation by β-glucan, iron binding capacity and radical scavenging ability of the β-glucan samples were evaluated. The β-glucan samples which showed better retardation of lipid oxidation also had higher iron binding capacity, which proved that the retardation of lipid oxidation by the β-glucan samples was related to their ability to chelate iron (Table 3; Study II, Figure 3a). The result of hydroxyl radical scavenging ability corresponded to the amount of phytate in the samples. The samples containing a higher content of

residual phytate had greater hydroxyl radical scavenging ability (Table 3; Study II, Figure 3b). Both iron binding capacity and hydroxyl radical scavenging ability of β-glucan samples decreased to a similar level when phytate was removed (Table 3; Study II, Figure 3. ab). Therefore, it was concluded that the retardation of lipid oxidation was more related to the residual phytate content rather than β-glucan (molecular weight and source) per se.

Table 3. The lipid oxidation, Fe(II) binding and hydroxyl radical scavenging of β-glucan samples containing residual phytate (with PA) and without phytate (without PA). The peroxide value and hexanal of β-glucan samples was calculated as percentage of the value for control emulsion after oxidation with 0.1 mM Fe(II) for 7 days. Data are shown as means ± standard deviation.

Measurements	**BBG-H**	**BBG-L**	**OBG-H**	**OBG-L**
M_w **g/mol**	466 000	204 000	355 000	247 000
Phytate content, %	0.04 ± 0.01	0.24 ± 0.02	1.23 ± 0.06	0.82 ± 0.01
Peroxide value, %				
with PA	62 ± 2	45 ± 5	53 ± 3	35 ± 4
without PA	94 ± 3	93 ± 3	117 ± 1	96 ± 1
Hexanal, %				
with PA	126 ± 6	38 ± 3	60 ± 4	21 ± 6
without PA	115 ± 15	105 ± 5	170 ± 21	126 ± 5
Fe(II) binding capacity, %				
with PA	4.8 ± 0.7	20.8 ± 0.7	16.8 ± 1.0	24.8 ± 2.0
without PA	3.6 ± 0.5	3.7 ± 0.2	4.6 ± 0.1	4.4 ± 0.2
HO· scavenging ability, %				
With PA	21.3 ± 0.3	29.0 ± 0.3	63.0 ± 0.2	52.6 ± 0.1
Without PA	19.0 ± 1.5	18.3 ± 1.0	18.3 ± 1.4	18.0 ± 3.0

Note: the concentration of β-glucan used for the measurement of lipid oxidation, Fe(II) binding capacity and hydroxyl radical scavenging ability was 0.56%, 0.6% and 0.6% (w/v), respectively.

5.3 Role of phytate in oxidative stability of β-glucan (III)

5.3.1 Oxidative stability of oat and barley β-glucan samples

In the presence of Fenton reagents, oat β-glucan was more stable than barley β-glucan, which was correlated to the higher content of residual phytate (1.23%) in the oat β-glucan samples compared to barley β-glucan (0.04%). The remaining viscosity of OBG-H was 83% after one-week oxidation with 0.1 mM Fe and 10 mM H_2O_2, while that of BBG-H was only 46% (Figure 11). Adding the same amount of phytic acid (0.2 mM) in BBG-H preserved 85% of its viscosity after one-week oxidation, similar to the viscosity of OBG-H.

5.3.2 Oxidative stability of oat β-glucan before and after phytate removal

The residual phytate in β-glucan samples contributed to the oxidative stability of β-glucan. No viscosity decrease was observed in OBG-H solution after storage with 0.1 mM Fe and 10 mM H_2O_2 for 24 hours (Figure 10. A). Only when more iron (0.5 mM and 1 mM) was added, was the degradation rate of OBG-H fast. However, when the residual phytate was removed, the oat β-glucan became vulnerable to degradation, with only 10% of viscosity and 23% of M_w remaining after 24 hours oxidation by 0.1 mM Fe and 10 mM H_2O_2 (Figure 10. B). Adding the same amount of phytic acid back to the phytate removed OBG solution protected the OBG from drastic degradation. This preserved over 80% of its viscosity and M_w after oxidation.

The M_w decrease of the β-glucan corresponded well with its viscosity drop after oxidation (Study III, Table 2), therefore the decrease in viscosity clearly indicated the degradation of β-glucan. The viscosity change of OBG-H within 3 hours was used for kinetic modelling of the β-glucan degradation because most of the viscosity drop occurred within 3 hours after the Fenton reagents were added. The best fitted model was the second order kinetic model with equation: $(1/V_t - 1/V_0) = kt$, $R^2 \geq 0.95$ (Study III, Table 1). The degradation constant k value was higher with OBG-H samples that had faster degradation rate. These samples were OBG-H with phytate removal or OBG-H oxidized with higher amount of Fe(II).

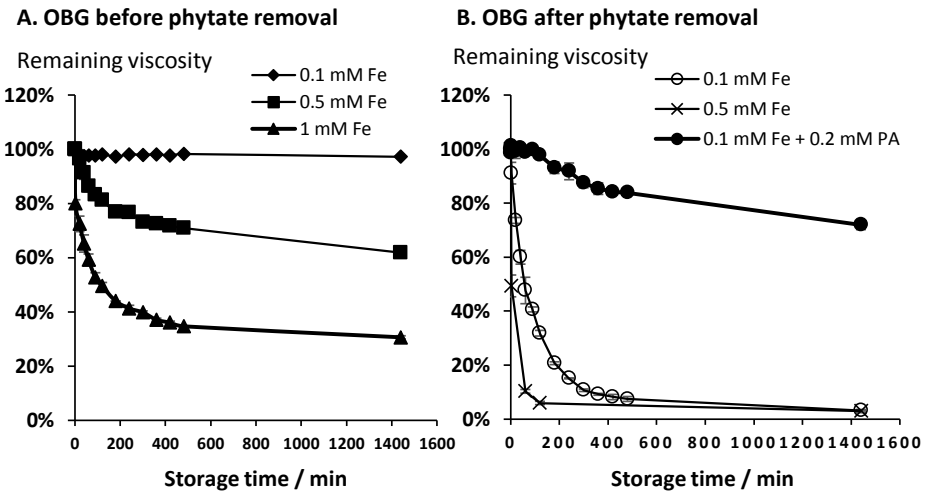

Figure 10. Oxidative degradation of oat β-glucan (OBG with high molecular weight) before (A) and after (B) phytate removal with different iron concentrations. All solutions contained 1.2% β-glucan and 10 mM H_2O_2. 0.2 mM phytic acid (PA) was added back to the phytate removed β-glucan in B.

5.3.3 Importance of phytic acid to iron ratio on oxidative stability of β-glucan

Higher ratio of PA:Fe preserved more viscosity during oxidation. Ratio of PA:Fe in 5:1 preserved 74% of the viscosity and ratio of PA:Fe in 2:1 preserved 46% of the viscosity after one-week oxidation. Lower ratio such as PA:Fe=2:5 did not show any protective effect. On the contrary, this ratio slightly enhanced the degradation of β-glucan. The remaining M_w was 45% which was even lower than the control sample (no PA addition, 47% of M_w remained).

5.3.4 Antioxidant effect of phytic acid in the presence of ascorbic acid

When ascorbic acid (0.5 mM) was added together with phytic acid (0.5 mM) in the BBG-H solution, the retardation effect of phytic acid was largely reduced with only 64.0% of M_w remaining. The addition of only phytic acid (0.5 mM) preserved over 90% of the M_w and viscosity after one-week of oxidation (Table 4). The addition of ascorbic acid itself enhanced the BBG-H degradation (remained 53% of original viscosity). More significant protection of β-glucan can be seen when a higher amount of phytic acid relative to the amount of ascorbic acid, was used. The remaining viscosity of BBG-H with 0.1 mM ascorbic acid and 0.5 mM phytic acid was 70.4% after one week.

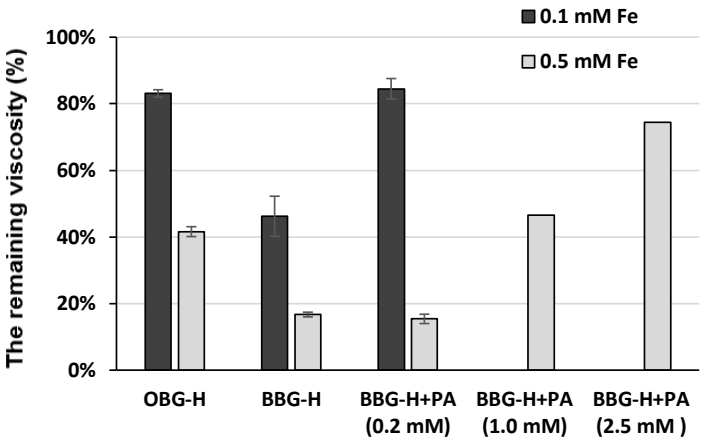

Figure 11. The remaining viscosity (%) of β-glucan solution after oxidizing with 0.1 mM or 0.5 mM Fe and 10 mM H_2O_2 for one week. The concentration of residual phytic acid (PA) in OBG-H solution was 0.2 mM. Error bars show standard deviation.

Table 4. The molecular weight and viscosity (in percentage) of barley β-glucan (0.8%, w/v) after oxidation with Fenton reagents (10 mM H_2O_2 + 0.1 mM Fe(II)) for 7 days in the presence or absence of ascorbic acid (AA) and phytic acid (PA). Data for viscosity are shown as means ± standard deviation.

Samples	Ascorbic acid (AA), mM	Phytic acid (PA), mM	Remaining M_w, %	Remaining viscosity, %
BBG-H	0	0	75.3	48.5 ± 1.0
BBG-H+AA	0.5	0	52.7	20.0 ± 4.0
BBG-H+AA+PA	0.5	0.5	64.0	29.0 ± 4.0
BBG-H+PA	0	0.5	93.4	90.0 ± 3.0

6 DISCUSSION

The oxidative degradation of cereal β-glucan induced by oxidized lipids was investigated with an oil-in-water emulsion model which is firstly discussed and then compared with the oxidative degradation of β-glucan in an aqueous system. Subsequently, the retardation of lipid oxidation by β-glucan and the involved mechanisms are discussed. Finally, the contribution of phytate to the antioxidant activity, oxidative stability and other possible properties of β-glucan are discussed.

6.1 Lipid oxidation induced degradation of β-glucan

Lipid oxidation induced significant degradation of β-glucan in the oil-in-water emulsion. The degradation extent varied depending on the extent of lipid oxidation, oil type, concentration of transition metal and the oxidation temperature. The pre-oxidized ML produced much more hydroperoxides than RO under the same oxidation conditions and the M_w decrease of β-glucan induced by ML was more than by RO. Fatty acids with a higher degree of unsaturation like methyl linoleate (ML) are more prone to oxidation than the monounsaturated oleic acids in rapeseed oil (RO). Obando et al. (2015) have also shown that more oxidized oil caused more co-oxidation of protein. Adding Fe(II) or oxidizing the samples at an elevated temperature caused significant lipid oxidation and β-glucan degradation. Similarly, the Fenton reaction induced degradation of β-glucan was also sensitive to the increase in Fe(II) concentration and oxidation temperature (Kivelä et al., 2011; Faure et al., 2013).

The co-oxidation of β-glucan during lipid oxidation was slower compared to the oxidative degradation of β-glucan in the aqueous system. When 1 mM Fe was used, the M_w decrease of β-glucan was up to 40% in the lipid oxidation system after 7 days (Study I) whereas 40% of M_w decrease was observed after oxidation with Fenton reagents for 1 day (Study III). In the study by Mäkelä et al. (2015), the M_w decreased was around 50% with 10 mM H_2O_2 and 1 mM Fe(II) after 1 day. Faure et al. (2015) have shown that adding only 1 mM Fe(II) to the barley β-glucan solution induced 35% of viscosity loss after 7 days. In their study, the pH of the solution was lowered to 4.5 in order to facilitate the Fenton reaction. In terms of the degradation rate, the rate of viscosity decrease of β-glucan was high at the beginning of the Fenton reaction induced degradation. Most of the degradation occurred within 3 hours with the Fenton reagents. Mäkelä et al. (2015) have also shown that the M_w of β-glucan dropped

dramatically in the first day and then levelled off later on when oxidized with Fenton reagents or ascorbic acid and Fe(II). In contrast to aqueous systems, a gradual and continuous degradation of β-glucan was seen in the lipid oxidation system. The viscosity and M_w of β-glucan continuously decreased with increased storage time.

Hydroxyl radicals (•OH) produced from the Fenton reaction are small, highly mobile, water soluble, and the most reactive species of activated oxygen (Ayala et al., 2014). In aqueous systems they can immediately attack the β-glucan molecules causing a viscosity drop. On the other hand, radicals formed from iron-catalyzed lipid peroxidation (e.g. LO• and LOO•) are larger and need to transfer from the oil phase through the oil droplet interface to reach the β-glucan molecules in the water phase (Figure 5; McClements and Decker, 2017; Schaich, 2005). Therefore, •OH is more detrimental to the β-glucan molecule than radicals originating from lipid oxidation. Oil-in-water emulsions containing smaller oil droplets have a larger surface area which can increase the interfacial area for reactions and facilitated the migration of lipid radicals (Lethuaut et al., 2002). This partly explains the higher extent of lipid oxidation and β-glucan degradation in the ML emulsion which had smaller oil droplets compared to the RO emulsion (Study II).

In our study, phase separation and formation of aggregates were observed in BBG-H emulsions (BBG-H + oil + Fe) when stored at 40ºC. The formed aggregates precipitated into the bottom of the reaction bottle, which explained the substantial viscosity drop in the sample. The aggregates were dissolved by the 0.01 M LiBr/DMSO solvent and measured in the analysis of M_w with HPSEC, therefore the M_w decrease was not as low as the viscosity decrease in this sample. Mäkelä et al. (2015) have observed the aggregates in oxidized β-glucan (by Fenton reagents) only with asymmetrical flow field-flow fractionation and suggested oxidation driven interactions between β-glucan molecules. They also showed that oxidation of β-glucan by Fenton reagents introduced internal carbonyl groups and produced smaller β-glucan molecules (Mäkelä et al., 2017). Li et al. (2011) showed that the smaller β-glucan molecules possessed higher diffusion rates, promoting the formation of aggregates. The formation of aggregates in the present study seemed to be also driven by oxidation because the precipitates were only seen in the β-glucan samples with the most degradation.

6.2 Role of β-glucan in retardation of lipid oxidation

β-Glucan is a neutral polysaccharide which may not be able to bind metal ions through electrostatic interactions. Polysaccharides containing functional side chains such as sulphated groups, carboxyl groups, uronic acid and sometimes peptides are able to bind metals through electrostatic interactions. Polyanionic polysaccharides such as pectin, xanthan gum and carrageenan have been shown to bind metals (Debon and Tester, 2001). In the present study, the β-glucan samples showed transition metal binding which was originated from the residual phytate in the β-glucan samples. This mainly explained the retardation of lipid oxidation by β-glucan. Zajdel et al. (2013) showed that even a small amount of phytate could inhibit lipid oxidation efficiently. When the phytate was removed, the transition metal binding, radical scavenging, and the retardation of lipid oxidation by all β-glucan samples was reduced to a similar level, indicating that the residual phytate is the key factor in this retardation effect.

The consumption of lipid oxidation intermediates e.g. radicals and simultaneous degradation of β-glucan was another mechanism in the retardation of lipid oxidation. BBG-H contained a low amount of phytate but showed significant retardation of lipid oxidation when 1 mM Fe was used. The β-glucan in this sample was simultaneously degraded. The lipid radicals or intermediates in lipid oxidation may interact with β-glucan, forming other stable compounds. In this way, β-glucan consumed the radicals, interrupting the propagation of lipid oxidation. Consumption of radicals and degradation of β-glucan also explained the hydroxyl radical scavenging ability of BBG-H shown in Study II (Figure 3b). Similarly, a study has shown that mannans and glucans exerted significant hydroxyl radical scavenging ability with a simultaneous degradation of the polysaccharides (Machova and Bystricky, 2013). The test of hydroxyl radical scavenging ability was based on stabilization/consumption of $^{\bullet}OH$ produced from the Fenton reaction. Therefore, the result of radical scavenging ability was attributed to the β-glucan which consumed the $^{\bullet}OH$ and the phytate in the β-glucan samples, suppressing the radical formation.

When a smaller amount of transition metal was used to accelerate the oxidation, the degradation of BBG-H was minimal, and the retardation of lipid oxidation was shown only in peroxide value but not in hexanal production. The decrease in the amount of peroxides in the emulsion was imparted mainly by the viscosity of the BBG-H solution. Shimada et al. (1996) have shown that the viscous solution of a polysaccharide slowed down the mobility of molecules such as oxygen and the reaction rate. The

added transition metal facilitated the decomposition of the lipid hydroperoxides located at the droplet surface (Nuchi et al., 2001) which further promoted the formation of secondary lipid oxidation products. This might explain the increase of hexanal amount in this BBG-H emulsion sample.

Studies have indicated that the molecular weight of polysaccharides played a role in their antioxidant activity (Matsumura et al., 2003). Low molecular weight polysaccharide have higher mobility which contributes to the improved antioxidant activity compared to high molecular weight polysaccharides. In the present study, it was observed that the emulsions containing low molecular weight β-glucan had better physical and oxidative stability compared to high molecular weight β-glucan. The physical stability of emulsions can contribute to its oxidative stability (Xu et al., 2017). Xu et al. (2017) also showed that when the phase stability of the emulsion was decreased by xanthan gum at pH 7, the lipid oxidation in the emulsion increased; and when the phase stability was increased by xanthan gum at pH 3.5, the lipid oxidation decreased (Xu et al., 2017). Burkus and Temelli (2000) have stated that β-glucan improved the physical stability of emulsions mainly by thickening the continuous phase of the emulsion and slowing down the coalescence of the oil droplets. However, in our study, the emulsions containing high M_w β-glucan were more viscous but less stable compared to emulsions containing low M_w β-glucan with lower viscosity. Lazaridou and Biliaderis (2009) also showed that β-glucan with long chains (at low amount) could cause bridging or depletion flocculation in emulsions. Phase separation of the emulsion would occur when a high amount of β-glucan was used, and this was due to the aggregation and gelation of β-glucan (Lazaridou and Biliaderis, 2009). The role of β-glucan molecular weight in the retardation of lipid oxidation was minimal, compared to the residual phytate in the β-glucan samples, because no retardation of lipid oxidation was seen after the phytate was removed from the β-glucan samples. This indicated that phytate associated with the β-glucan sample can overwhelm the role of β-glucan structural features with respect to antioxidant activity. Wrong conclusions would have been made if the phytate in the sample was overlooked.

6.3 Role of phytate in oxidative stability of β-glucan

6.3.1 Improved oxidative stability of β-glucan by phytate

To induce fast and more significant oxidative degradation of β-glucan, the Fenton reaction instead of lipid oxidation was used to study the role of phytate in the oxidative stability of β-glucan. Oat β-glucan was more stable than barley β-glucan under oxidative conditions. Mäkelä et al. (2017) and Faure et al. (2015) also showed that oat β-glucan had degraded less than barley β-glucan when oxidizing with either Fenton reagents or ascorbic acid and Fe. Faure et al. (2015) have suggested that oat β-glucan had a stronger affinity for iron(II) therefore the formation of hydroxyl radicals was less in the oat β-glucan solution compared to the barley β-glucan solution when adding Fenton reagents. According to our study, the iron binding ability of oat β-glucan was mostly from the residual phytate in the sample because the iron binding ability of both oat and barley β-glucan was reduced to a low level when the phytate was removed from the β-glucan sample. Furthermore, oat β-glucan became as vulnerable as barley β-glucan when the residual phytate in oat β-glucan was removed.

The added phytic acid considerably retarded the oxidative degradation of the phytate-removed oat β-glucan but not as much as the original residual phytate in the β-glucan sample. Unlike the residual phytate which is located close to the β-glucan molecule, the added phytic acid may not have a similar interaction with β-glucan and therefore provided less protection. Better protection can be expected when the iron is chelated by phytate close to the β-glucan molecule. Additionally, the anion exchange and dialysis, used to remove phytate, likely removed other molecules such as proteins and cations. The intrinsic minerals or other compounds in original β-glucan may play a role in the chelation ability and antioxidant activity of phytate.

6.3.2 Role of phytic acid to iron ratio in iron-catalyzed oxidation

It is well known that phytic acid (PA) has antioxidant activity by chelation of transition metals (Graf and Eaton, 1990). The ratio of PA to minerals determines the saturation degree of phosphorus groups with cations, which would affect the solubility, availability to bind extra cations and furthermore the antioxidant activity of phytate (Graf 1983; Champagne 1985). Rimbach and Pallauf (1998) have reported that the inhibition of hydroxyl radicals via the Fenton reaction requires a ratio of PA to minerals > 5:1. In our study, significant retardation of lipid oxidation and oxidative degradation of β-glucan were shown when the ratio of PA to Fe(II) was 2:1 and 5:1. However, when the ratio of PA to Fe(II) was 2:5, the oxidative degradation of β-glucan was not inhibited but even enhanced. As a chelator, phytate was able to block all coordination sites in iron and efficiently prevent the iron from acting as pro-oxidant. However, when the iron concentration was too high the phytate was fully occupied, and the excessive unbound Fe(II) was still able to catalyze the Fenton reaction. The chelated iron is catalytically active if the iron within the complex retains an available coordination site (Graf et al., 1984). Metal chelators can also be pro-oxidants at certain ratios with transition metals. For example, Frankel et al. (2002) have shown that EDTA enhanced oxidation at a low ratio of EDTA to Fe in emulsions. Huang et al. (2016) have used molar ratio of EDTA:$FeSO_4$ in 1:1 to accelerate the oxidation of peptides.

Earlier studies on the ratio of phytic acid to minerals were mostly in model systems with added pure phytic acid (Graf et al., 1984). We showed that the intrinsic ratio of phytic acid to minerals is also important in the antioxidant effect. The phytate content was higher in OBG-H compared to that in OBG-L, while the retardation of lipid oxidation and the iron binding ability was less in OBG-H. The mineral content (e.g. Ca, Mg, Fe, Mn) in OBG-H was also higher than in OBG-L which was attributed to the high content of phytate. The intrinsic transition metals such as Fe and Mn contributed to the oxidation reactions. Kivelä et al. (2009) and Mäkinen et al. (2012) have shown that the intrinsic Fe was enough to cause significant oxidative degradation of β-glucan. Moreover, the high amount of minerals in OBG-H largely occupied the chelating position of phytate and reduced its chelating power.

6.3.3 Reduction of antioxidant effect of phytic acid by ascorbic acid

Ascorbic acid has been shown to induce the oxidative degradation of β-glucan through Fenton type oxidation (Kivelä et al., 2009; Faure et al., 2013; Mäkelä et al., 2015). The addition of phytate significantly decreased the radical formation but could not prevent the viscosity loss of the β-glucan extract treated with ascorbic acid (Kivelä et al., 2009; Faure et al., 2012). In our study, the phytic acid significantly retarded the degradation of β-glucan induced by the Fenton reaction. However, when the ascorbic acid was added, the retardation of oxidation by phytic acid was largely weakened and even totally counteracted.

Phytic acid is able to chelate transition metals and it also causes a substantial shift in the redox potential of iron from Fe(II) to Fe(III) without production of $^{\bullet}OH$ in the presence of oxygen (Graf et al., 1987). Compared to Fe(II), Fe(III) is less soluble and inert in oxidation even in the presence of oxygen and polyunsaturated lipids. On the contrary, ascorbic acid reduces Fe(III) to Fe(II) which then accelerates the Fenton reaction and radical formation (Teucher et al., 2004). The competition between ascorbic acid and phytic acid in shifting the redox potential of iron is presented in Figure 12. Moreover, ascorbic acid can acidify the solution and form soluble iron-ascorbate complexes, which keeps iron soluble over the pH range 2-11(Lopez et al. 2002; Nielsen et al., 2013) and this therefore affects the metal binding capacity of phytate (Persson et al., 1998). Phytic acid-mineral chelates are insoluble at neutral pH. When the pH decreases, the complex between phytic acid and metals becomes soluble (Persson et al., 1991; Persson et al., 1998), and the ability of phytic acid to retard oxidation is reduced. In fact, Faure et al. (2015) observed that the oat β-glucan sample did not inhibit the hydroxyl radical formation when the pH dropped to 2.7. This was very likely due to the decrease in the chelation ability of phytate in the β-glucan sample at low pH.

Studies have shown that ascorbic acid added in the diet could overcome the effect of phytic acid on the chelation of non-haem Fe (Hurrell and Egli, 2010). Similarly, the ascorbic acid likely reduced the ability of phytic acid to bind iron, therefore counteracted the protective effect of phytic acid in the present study. The inhibition of oxidation by phytic acid was shown only when high amount of phytic acid (0.5 mM) was used (compared to 0.1 mM ascorbic acid). Lee and Hendricks (1997) also reported that the inhibition of iron-ascorbate induced lipid peroxidation by phytic acid

was only seen when the ascorbic acid was added in low concentrations. Thus, other competitors for iron complexation can reduce the antioxidant effect of phytic acid.

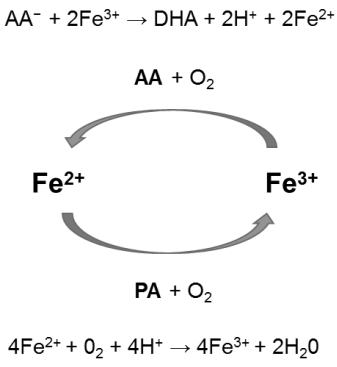

Figure 12. The competition between ascorbic acid (AA) and phytic acid (PA) in shifting the redox potential of iron. AA reduces Fe^{3+} to Fe^{2+} which facilitates the Fenton reaction. PA oxidizes Fe^{2+} to Fe^{3+} without production of $^{\bullet}OH$, and therefore hinders the participation of Fe^{2+} in Fenton reaction.

6.4 Contribution of phytate to other properties of β-glucan

As is well known, both phytate and β-glucan are located in the aleurone layer of the grains: phytate is found inside the aleurone cells but β-glucan is in the cell walls. In plants, ascorbic acid induced oxidative scission of plant cell wall polysaccharides has been shown (Fry, 1998). It is known that *myo*-inositol is a substrate for the cell wall polysaccharide formation (Reddy et al., 1989) but whether phytate plays a role in regulating the oxidation of cell wall polysaccharides is not clear in plants. Interaction between phytate and β-glucan may occur during the extraction of β-glucan because the phytate content in whole grain is around 1% (Table 1) and the content can be up to 6% in the β-glucan extract (Kivelä, 2011). Oat and barley grains contain similar amounts of phytate, but higher amounts of phytate were present in oat β-glucan samples probably due to the thermal treatment of oat material which hinders the further enzymatic degradation of phytate during extraction. Studies have been using cereal β-glucan extracts from various sources and under different extraction conditions. It is very likely that the β-glucan materials contain various amount of β-glucan and impurities. The derived or associated substances in the β-glucan materials may be responsible for some of the effects and need to be identified (Chen and Raymond, 2008).

In our study, the residual phytate content was higher in oat β-glucan which imparted oat β-glucan better antioxidant activity and oxidative stability than barley β-glucan. The residual phytate may contribute to other physico-chemical properties of β-glucan. Ghotra et al., (2007) have found that the presence of more negatively charged phosphorus substitutions in oat β-glucan contributed to its better extractability and solubility compared to barley β-glucan. In the present study, it was observed that after phytate removal the β-glucan could not dissolve as well as the original β-glucan, although the solubility may also be affected by the drying. As reviewed, phytic acid has some physiological effects such as blood cholesterol lowering (Jariwalla et al., 1990; Lee et al., 2007) and blood glucose management (Yoon et al., 1983; Schlemmer 2009) which are found with β-glucan as well. Therefore, the contribution of phytate to the physiological effects of β-glucan containing foods needs to be considered.

7 CONCLUSIONS

The stability of cereal β-glucan is of importance to its physico-chemical and physiological functionalities. The oxidative degradation of β-glucan caused by hydroxyl radicals has been studied in an aqueous system. This study sheds light on the role of lipid oxidation in the oxidative stability of β-glucan in multi-phased systems. Furthermore, high phytate content could be expected in the oat β-glucan sample and the contribution of the residual phytate to the antioxidant activity and oxidative stability of β-glucan was studied.

Lipid radicals formed from lipid oxidation induced significant degradation of β-glucan, especially in the presence of iron and elevated temperature (40°C). The degradation of β-glucan induced by lipid oxidation was milder than that induced by the Fenton reaction. The oil type and oxidation extent as well as the amount of transition metal affected the degradation. The β-glucan added to the emulsions was found to retard the lipid oxidation. The oat β-glucan showed better capacity in the retardation of lipid oxidation than barley β-glucan, and the β-glucan with lower molecular weight retarded lipid oxidation to a greater extent. The reason was found to be the higher content of phytate in the oat β-glucan samples that contributed to the improved retardation of lipid oxidation, compared to barley β-glucan samples. The higher iron binding capacity and hydroxyl radical scavenging ability in oat β-glucan samples was also related to the high phytate content in oat β-glucan. The antioxidant activity of all samples was declined to a similar level when phytate was removed from the sample, proving that it was the phytate, instead of the differences in β-glucan source and molecular weight that played a major role in the antioxidant activity of the sample.

The presence of higher residual phytate also explained the improved oxidative stability of oat β-glucan compared to barley β-glucan. The degradation of barley β-glucan can be controlled by adding phytic acid to the β-glucan although the ratio of phytic acid to iron and the presence of other chelators such as ascorbic acid should be considered.

This study advances the understanding of β-glucan stability in complex systems related to foods and indicates that the bioactive components such as phytate associated with dietary fiber can have a major contribution in fiber related reactions and functionalities in food and nutrition.

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