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Retardation of oxidation by residual phytate in purified cereal β-glucans

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ABSTRACT

Polysaccharides stabilize emulsions against phase separation and oxidation. This study focused on the role of molecule weight, origin, and residual phytate in the antioxidant activity of β-glucans. The antioxidant activity was investigated by measuring the retardation of lipid oxidation, iron binding capacity and hydroxyl radical scavenging ability of purified oat and barley β-glucans. Low molecular weight β-glucan stabilized the emulsions against phase separation and oxidation better than high molecular weight β-glucan. The oxidative stability of the emulsions, however, correlated to the content of residual phytate in the β-glucan samples. Oat β-glucans which contained higher amount of residual phytate showed higher antioxidant activity than barley β-glucans. When phytate was removed from the β-glucan samples by ion exchange, the antioxidant activity of all β-glucans was reduced to the same level. The study therefore showed that residual phytate played a major role in the antioxidant activity of cereal β-glucans. The study highlights that even at low concentration, phytate content should be considered when evaluating the antioxidant effect of plant polysaccharide extracts.

1. Introduction

Cereal β-D-(1 → 3), (1 → 4)-glucan is a linear and soluble non-starch polysaccharide. Oats and barley are the most relevant sources of cereal β-glucan with the content varying from 3% to 7% and from 3% to 11%, respectively (Cui & Wood, 2000). In these cereals, β-glucan is located in endospermic and aleuronic cell walls. Much attention has been given to β-glucan due to its health and technological benefits that are generally related to the ability of β-glucan to form highly viscous shear-thinning solutions even at low concentrations (Lazaridou & Biliaderis, 2007). β-Glucan, as a food hydrocolloid, is capable of improving the phase stability of emulsions and foams by increasing the viscosity of the continuous phase and forming a gelled network (Burkus & Temelli, 2000).

Cereal β-glucan is susceptible to degradation due to the presence of enzymes, heat, mechanical forces or acids, leading to viscosity and molecular weight (Mw) decrease (Kivelä, 2011). In addition, oxidative degradation of cereal β-glucan has been shown in the presence of ascorbic acid and iron which form hydroxyl radicals to attack β-glucan (Faure, Munger, & Nyström, 2012; Kivelä, Gates, & Sontag-Strohm, 2009; Mäkelä, Maina & Sontag-Strohm, 2015). Radicals formed from lipid oxidation have also been found to induce degradation of barley β-glucan (Wang, Mäkelä, Maina, Lampi, & Sontag-Strohm, 2016). The tendency to degradation however, was shown to be different for oat and barley β-glucans (Faure, Koppenol, & Nyström, 2015). Less degradation was found in oat β-glucan than in barley β-glucan, and this was explained by the higher iron binding ability of oat β-glucan, which slowed down the iron catalysed oxidative reactions such as hydroxyl radical

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formation in Fenton reaction. Nonetheless, the mechanism for β-glucan to bind metal is not clear. More evidence is needed to understand the differences and mechanisms of oat and barley β-glucan oxidation.

Recently, barley β-glucan has been shown to enhance the oxidative stability of emulsions. Barley β-glucan retarded hexanal production during lipid oxidation in an emulsion model system (Wang et al., 2016). Polysaccharides are able to stabilize oxidative reaction in emulsions through multiple mechanisms including viscosity enhancement, transition metal binding and free radical scavenging (Shimada, Okada, Matsuo, & Yoshioka, 1996). By enhancing the viscosity of a solution, oxygen diffusion rate and oil droplet collision probability are decreased, therefore hindering lipid oxidation. Transition metal binding is usually a typical feature of polyanionic polysaccharides such as pectin, xanthan gum and carrageenan (Debon & Tester, 2001). These polysaccharides contain functional side chains such as sulphated groups, carboxyl groups, uronic acids and sometimes peptides that enable them to bind metals through electrostatic interactions. Matsumura et al. (2003) have shown that soluble soybean polysaccharide and gum arabic had inhibitory effect on lipid oxidation in emulsions due to the covalently-attached peptide moieties in the polysaccharides, whereas pullulan and maltodextrin which lack peptide moieties had no inhibitory effect. Unlike other polyanionic polysaccharides, β-glucan is a neutral polysaccharide which has lower tendency to bind metals via electrostatic interactions.

Free radical scavenging activity of polysaccharides has been reported. Mannans prepared from the cell wall of yeasts and glucans from algae and plants have been shown to scavenge free radicals such as hydroxyl radicals which also results in their simultaneous degradation (Machova & Bystricky, 2013). The retardation of oxidation by β-glucan was related to their oxidative degradation which consumed the free radicals and therefore decreased their availability for other oxidation reactions (Machova & Bystricky, 2013; Wang et al., 2016). Low molecular weight polysaccharides have higher mobility and therefore are thought to be more effective in the antioxidant activity compared to larger polysaccharides (Matsumura et al., 2003). Błaszczyk et al. (2015) have shown that diet supplementation with oat β-glucan improves stress oxidative parameters in spleen, with low Mw β-glucan being more effective.

Polysaccharide extracts usually contain various amounts of impurities depending on the raw material, extraction and purification methods which can contribute to the overall antioxidant activity of the polysaccharide. Kivelä (2011) has reported that β-glucan extracted from oat bran contained 40% of β-glucan, other polysaccharides (18%), proteins (15%), phytic acid (5%) and various minerals. Higher β-glucan content was obtained by including an ethanol precipitation step which increased the β-glucan content to 80–90% (Kivelä, 2011) and reduced protein to 4% and phytic acid to about 2% dw. In another study with whole oat flour as raw material, small amounts of fat (0.7–1.0%), protein (4.7–5.7%), starch (1.3–2.1%), pentosan (1.0–1.5%) and mineral (ash, 1.3–1.6%) were shown to be extracted along with β-glucan when using acid, alkaline and enzymatic extraction methods (Ahmad, Anjum, Zahoor, Nawaz, & Ahmed, 2010). This study reported that phosphorous, potassium, magnesium and calcium appeared as major minerals in β-glucan extracts, while iron, manganese and copper were minor.

When using such extracts in the stabilization of emulsion and oxidation studies, the presence of impurities should be taken into consideration. In oxidation studies, the presence of proteins or phytic acid may lead to a change in oxidation kinetics. Especially, phytic acid is a well-known natural antioxidant which is able to complex various bi- and trivalent cations such as iron. This strongly facilitates the oxidation of Fe (II) to Fe (III) thus inhibiting hydroxyl radical production in the Fenton reaction (Rimbach & Pallauf, 1998). Phytate may also reduce the iron mediated lipid peroxidation and lipid autoxidation (Graf & Eaton, 1990; Zajdel, Wilczok, Węglarz & Dzierzewicz, 2013).

Several studies have reported the presence of phytate in β-glucan extracts. Nonetheless, the role of residual phytate in the antioxidant activity of the purified β-glucan (>94% purity) has not been evaluated. Moreover, the mechanisms of β-glucan to bind iron and thus retard oxidation should be examined. The aim of this study was to evaluate the retardation of oxidation by high and low Mw β-glucans purified from oat and barley, and to further investigate the role of residual phytate in the antioxidant activity of purified β-glucan.

2. Material and methods

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).

2.1. Characterization of mineral contents in β-glucan raw material

Mineral content (P, Ca, Mg, K, Fe, Mn, Zn and Cu) of the β-glucan samples were determined by ICP-OES (Thermo Scietific iCAP 6000). For this analysis, samples were first dried at 100 °C for 24 h after which 500 mg of each sample was washed with 1 N HNO_3 (Merck Tracepur) and H_2O_2 (30%, Merck, Germany). Subsequently, the samples were digested by adding 3 ml of 1 N HNO_3 and 1 ml of 30% H_2O_2, using a microwave digestion system (CEM MARSXpress) in closed vessels. The heating program was as follows: heating to 70 °C in 20 min, heating to 130 °C in 40 min, heating to 170 °C in 30 min, and then holding at 200 °C for 40 min. After cooling, the digested samples were diluted with 0.02 N HNO_3 to a volume of 25 ml before ICP-OES measurements. The minerals were quantified using external standard curves. Each mineral was measured at two or four different wavelengths depending on the mineral type. The analysis was carried out in duplicates and a certified reference sample (NIST 1567) was used to ensure the accuracy of the method.

2.2. Preparation of β-glucan solutions

β-Glucan solutions (0.7%) were prepared as previously described (Wang et al., 2016). After weighing, the β-glucan samples were first wetted with 99.5% ethanol (8% of the final volume). MilliQ-water (Millipore system, Merck, Millipore, Germany) was added up to 80% of the total volume and solution was stirred at 85 °C for 2 h. After cooling down, the volumetric flasks were filled to the mark and stirring was continued for 1 h at room temperature.

2.3. Phytate removal

Phytate was removed by using ion exchange resins as described by Kumagai, Ishida, Koizumi, and Sakurai (2002) with some modifications. The resins (Amberlite IRA-410 Chloride form 20–25 mesh, Sigma-Aldrich, Saint Louis, USA) were first activated by successively washing with 1 N HCl, deionised water, 1 N NaOH, deionised water, 1 N HCl, deionised water, and 1N HCl prior to use. The resin and 0.3% of β-glucan (w/v) solution (pH adjusted to 4 with HCl) were mixed and stirred at 4 °C for 2 h. The mixture of β-glucan and resin was then filtered using a cotton cloth, and the filtrate (β-glucan) was dialysed against distilled water overnight prior to freeze-drying. The phytic acid content of the samples before and after phytate removal was measured as phosphorus released by

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