Antibacterial and antioxidant properties of mixed linkage beta-oligosaccharides from extracted β-glucan hydrolysed by Penicillium occitanis EGL lichenase

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ABSTRACT
The aim of this study was first to ascertain the chemical composition and the physicochemical properties of cereal extracted β-glucan from barley flour. Secondly, to assess the antioxidant properties and the antibacterial properties of extracted β-glucan hydrolysates. The proximate composition, FT-IR and scanning electron microscopy of extracted β-Glucan were studied. Hydrolysates from extracted β-glucan, obtained by lichenase EGf from Penicillium occitanis, were a mixed linkage beta-oligosaccharides (MLBO) of trisaccharides and tetrasaccharides. MLBO showed a DPPH radical scavenger with IC50 about 1.8 ± 0.01 mg/mL whereas the IC50 of extracted β-glucan was about 5 ± 0.01 mg/mL. MLBO showed a high antioxidative capacity (175 μmol/mL α-tocopherol equivalents) at 5 mg/mL. The antimicrobial activity was confirmed against all tested bacteria especially at 20 mg/mL of MLBO while no inhibition was observed for all the strains used after the addition of either EGf or extracted β-glucan.

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1. Introduction

The mixed linkage (1-3)(1-4)-β-d-glucan (β-glucan) of cereals possesses a number of functionalities and roles that make it unique as a plant cell wall component and as a dietary fibre (Wood 2007). Extraction is one of the most widely used unit operations in the food industry. It is mainly used for obtaining certain desired components initially retained in a food matrix (Pinelo et al. 2005). Molecules obtained by extraction may be used as food additives or for exerting peculiar beneficial effects on human health (Delgado-Adámez et al. 2012). In terms of β-glucan extraction, a great variety of approaches based on different principles have been developed. For instance, alkaline extraction, acidic extraction, hot water extraction and enzymatic method extraction have been applied to extract β-glucan from barley (Asif et al. 2010). Beta-glucans, the major fibre constituents of barley, have been implicated in lowering plasma cholesterol, improving lipid metabolism and reducing glycaemic index (Keenan et al. 2007). Numerous reports have indicated that β-glucans have an activity to stimulate immunity and decrease infectious complications in humans (Dellinger et al. 1999; Talbott & Talbott 2009) and animals (Murphy et al. 2008) and are also known to activate macrophages, neutrophiles and NK cells by binding to the β-glucan receptor on the cells (Kogan 2000; Mantovani et al. 2008). Other general activities of β-glucan are anti-inflammatory effect and anti-tumour activity (Ruthes et al. 2013; Wang et al. 2014). Degradation of β-glucans in nature is catalysed by β-glucanases, which can be grouped into four main categories according to the type of glycosidic linkage they cleave: β-1,3-1,4-glucanases (lichenases, EC 3.2.1.73), β-1,4-glucanases (cellulases, EC 3.2.1.4), β-1,3-glucanases (laminarinases, EC 3.2.1.39) and β-1,3(4)-glucanases (EC 3.2.1.6) (Boyce & Walsh 2007; Yoo et al. 2007). Lichenase is an endo-β-1,3–1,4-glucanase that cleaves β-1,4 linkages adjacent to β-1,3 bonds in lichenan or barley β-glucan, yielding chiefly 3-O-β-cellobiosyl-d-glucose and 3-O-β-cellotriosyl-d-glucose (Anderson & Stone 1975; Fleming & Kawakami 1977). It has been shown that β-glucan oligosaccharides have the ability to modulate the immune system in humans, animals and fish (Otaka 2006). Oligosaccharides, which have a physiological function such as low cariogenicity (Day & Misook 2011), bifidobacteria growth factor (Jaskari et al. 1998) and modulation of the immune system with saccharides and oligosaccharides (Otaka 2006). Many studies examined the effect of laminarin or lichenan oligosaccharides hydrolysed by an endo-1,3-β-glucanase (Pang et al. 2005), synthetic oligosaccharides (Wang et al. 2010) and yeast oligosaccharides prepared by autolysis combined with enzymatic digestion (Lee et al. 2002; Kim et al. 2005) on the improvement of immune efficiency, and they are expected to maintain intestinal health as ‘prebiotics’ for humans and animals (Otaka 2006). The development of oligosaccharide production requires the identification of new enzymes and application technology of these enzymes because these oligosaccharides are produced from respective polysaccharides or sugars by enzymatic hydrolysis and/or transfer reaction. In previous work, Penicillium occitanis (Pol6) was screened out in our laboratory which exhibited an excellent capacity to produce lichenase (Chaari et al. 2014). To our knowledge, there is no information about the biological activities of oligosaccharides from barley flour β-glucan hydrolysed by lichenase. This work aimed to the production of mixed linkage-oligosaccharides from extracted β-glucan by enzymatic hydrolysis. The antioxidant and antibacterial activities of barley flour extracted β-glucan hydrolysates, prepared by treatment with lichenase from P. occitanis, were evaluated.
2. Results and discussion

2.1. Chemical composition of barley flour and extracted barley β-glucan

β-glucan was extracted from barley flour with a yield of 3.6%. This yield value was well comparable with those reported by Izydorczyk and Dexter (2008) which ranges from 2.8 to 11%. However, the yield level of extracted β-glucan was higher than that of rye (1.2–2%) and wheat (0.4–1.4%) (Lazardiou et al. 2007). The content of β-glucans in barley is influenced by both genetic and environmental factors and the interactions between the two (Andersson et al. 1999). Table S1 summarises the general composition of barley flour and extracted barley β-glucan. Barley flour contains, on average, 10.26% moisture, 1.23% ash, 8.73% protein, 1.59% lipid and 33.75% total dietary fibre. These results are lower with the earlier findings reported for Canadian varieties by (Li 2010). Helm and Francisco in 2004 also concluded that Brazilian barley varieties showed crude protein content from 11.55 to 15.92%, crude fat 2.91–4.00%, ash 1.51–2.27% and crude fibre 5.95–7.12%. The dietary fibre of barley flour in this study was found 2.91% soluble, 30.84% insoluble and 34% total dietary fibre. In earlier studies, the variations in total dietary fibre, soluble dietary fibre and insoluble dietary fibre content of barley flour have been reported ranging from 7.5 to 16.8%, 5.6–6.4%, and 1.9–10.4%, respectively, in barley (Vasanthan & Temelli 2002; Helm & De Fransisco 2004).

It can also be seen in Table S1 that starch was the largest dominating impurity in the extracted β-glucan. β-glucan contain moisture and ash about 6.55 and 10.41%, respectively. Only very low protein and lipid contents in the extracted β-glucan were obtained. These values are lower than those reported in barley fibre concentrate, which was obtained from Ceba Foods (Lund, Sweden) (5.04 g vs. 8 g per 100 g of dry weight for protein and 0.036 g vs. 10 g per 100 g of dry weight for lipid). Mineral composition of extracted β-glucan showed that phosphorus was the predominant mineral component (19 mg/g of dry weight). According to Asif et al. (2010), potassium was the abundant mineral in the extracted β-glucan. The others minerals for extracted β-glucan (Mg, Ca, Na, K and Fe) were also found to be 8.45, 6.4, 5.25, 4.55 and 0.5 (mg/g of dry weight), respectively. These values were higher than those obtained by Asif et al. (2010). These minerals have a capacity to modify functional properties of dietary fibre and offer a buffering action in the solutions.

2.2. FT-IR analysis

The FT-IR spectra of carbohydrates are used for determination of their structural features. The wave number between 950 and 1200 cm\(^{-1}\) is often called the fingerprint of molecules because it allows the identification of major chemical groups in polysaccharides: the position and intensity of the bands that are specific for each polysaccharide (Kacuráková & Wilson 2001; Cernà et al. 2003). A FT-IR spectrum of extracted β-glucan was compared against commercial β-glucan. Figure S1 indicates that extracted β-glucan had an absorption peak at 1074 cm\(^{-1}\), which is due to the stretching of (CC) and (CO) groups and represents the presence of glucopyranose. Absorption peak at 1074 cm\(^{-1}\) and peaks at 1156–1165 cm\(^{-1}\) indicate the linear structure of β-glucan linked through (1→3) linkage. It is also important to note that the spectra showed absorption peak at 896 cm\(^{-1}\) that is indicative of linked glycosidic bonds, and no absorption near 840 cm\(^{-1}\) showed the absence of α-linked glycosidic bonds (Wang et al. 2005). The spectra were similar for commercial and extracted β-glucan.
2.3. **Scanning electron microscopy**

In order to compare the structural integrity of the extracted and commercial β-glucan, scanning electron microscopy was applied. Figure S2 shows that the extracted and commercial samples had similar morphology. The extracted β-glucan showed in addition some granules appearance of starch. The structures of the samples were regular and comprised granular particles with numerous aggregates with modular shape.

2.4. **Production of β-glucan oligosaccharides**

In this work, lichenase EG₇ from *P. occitanis* was chosen for its specificity and high thermostability. The lichenase EG₇ was used, as described in the experimental part of the supplementary material, for the production of β-glucan oligosaccharides. The TLC analysis of products generated during extracted β-glucan hydrolysis by EG₇ is shown in Figure S3. After 16 h of incubation, the major products obtained from extracted β-glucan hydrolysis by EG₇ were a mixed linkage beta-oligosaccharides (MLBO): trisaccharides and tetrasaccharides. This result was in agreement with (Wood et al. 1994; Izydorczyk et al. 1998) who found that endo-β-1,3-1, 4-glucanases (lichenases) cleave β-1,4 linkages adjacent to β-1,3 bonds in glucans, yielding chiefly 3-O-β-celllobiosyl-d-glucopyranose (DP3) and (3-O-β-cellotriosyl-d-glucopyranose (DP4) (Chaari, Bhiri, et al. 2012; Chaari, Blibech, et al. 2012).

2.5. **Determination of the antioxidant activity of the MLBO**

In this study, the antioxidant activities of MLBO was investigated and compared with that of BHA, a widely used synthetic antioxidant.

2.5.1. **DPPH radical-scavenging activity**

DPPH is a stable free radical that shows maximum absorbance at 517 nm. When DPPH radicals encounter a proton-donating substrate such as an antioxidant, the radicals would be scavenged and the absorbance is reduced (Shimada et al. 1992). The decrease in absorbance is taken as a measure for radical scavenging. Thus, the DPPH radicals were widely used to investigate the scavenging activity of antioxidant compounds. Figure S4A shows the DPPH radical-scavenging activity of BHA, MLBO, extracted β-glucan and EG₇ at various concentrations. The results clearly indicated that EG₇ showed no radical-scavenging activity even when increasing its concentration. Moreover, extracted β-glucan showed a lower radical-scavenging activity than BHA at the same concentration. The MLBO DPPH radical-scavenging activities were concentration-dependent; the values increased with increasing MLBO concentration. According to Bougatef et al. (2009), the concentration of 50% inhibition (IC50) of the lowest DPPH radical corresponds to the inhibitory capacity of the higher free radical-scavenging ability. MLBO showed a DPPH radical scavenger with IC50 about 1.8 ± 0.01 mg/ml whereas the IC50 of extracted β-glucan was about 5 ± 0.01 mg/ml. The data obtained reveal that the MLBO are free radical inhibitors and primary antioxidants that react with free radicals.

2.5.2. **Total antioxidant capacity**

In the phosphomolybdenum assay, which is a quantitative method to evaluate the antioxidant capacity (Arabshahi-Delouee & Urooj 2007), all samples exhibited different degrees of activity as shown in Figure S4B. The total antioxidant activity increased with the concentration of BHA and MLBO generated and the total antioxidant activity of extracted β-glucan
extract is lower than MLBO. In fact, the concentration of α-tocopherol equivalent is about six times greater for MLBO when compared with the β-glucan extract at a concentration of 5 mg/ml. The BHA used as a positive control reached its maximum antioxidant activity (190.5 μmol/mL α-tocopherol equivalents) from a concentration of 2.5 mg/mL. MLBO showed the greatest antioxidative capacity (175 μmol/mL α-tocopherol equivalents) at 5 mg/ml. While EG showed no antioxidant activity in this experiment which allows us to deduce that the most important antioxidant activity is mainly due to MLBO released.

2.6. Antibacterial activity

Antibacterial activities of samples at a concentration of 10 and 20 mg/mL are summarised in Table S2. The results showed that the most important inhibition zone were detected in E. coli and B. subtilis strains and it is observed whatever the MLBO concentration used (10 or 20 mg/mL). We showed also that there was no inhibition zone to be observed for all the strains used after the addition of either EG or β-glucan extract. Among all the bacteria tested, there was no inhibition zone to be observed when bacteria including B. thurigenesis. For strains Actinomycete sp., S. typhimurium and P. aeruginosa, the inhibition zones were detected when MLBO was used at 20 mg/mL (Figure S5). Strains of P. aeruginosa and K. pneumoniae are resistant to the action of ampicillin as shown in Figure S5. This resistance is provided by a β-lactamase enzyme expressed in this strain which hydrolyses the antibiotics belonging to the family of β-lactam antibiotics including ampicillin part (Rolinson 1974). Despite this resistance, inhibition was detected in these two pathogenic strains by MLBO at a concentration of 20 mg/mL.

3. Conclusion

In this study, barley flour extracted beta-glucan was subjected to enzymatic hydrolysis for the production of mixed oligosaccharides (MLBO). The major hydrolysis products of extracted beta-glucan are oligosaccharides with DP 3 and DP4 which found to possess antioxidant and antibacterial activities. Therefore, MLBO can be used as a promising candidate material in the food industry.

Supplemental data and research materials

Experimental materials relating to this article are available online http://dx.doi.org/10.1080/14786419.2015.1056185, alongside Figure S1–S5 and Tables S1, S2.

Disclosure statement

No potential conflict of interest was reported by the authors.

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