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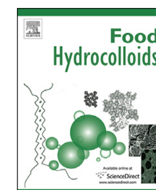

Structural and functional characterization of oxidized feruloylated arabinoxylan from wheat

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ABSTRACT

Chemical and enzymatic modifications of carbohydrates offer a promising way of producing new fibre-rich food ingredients since these methods modify the functionality of carbohydrates. Hydroxyl radical oxidized and oxidatively crosslinked (peroxidase/hydrogen peroxide) feruloylated arabinoxylan (AX) isolates were prepared and examined for structural and functional properties. It was demonstrated that hydroxyl radical oxidation provokes polymer degradation: considerable decrease in molecular size was observed with size-exclusion HPLC. Neither a change in the arabinose/xylose ratio nor in the ferulic acid content could be observed upon hydroxyl radical oxidation. Crosslinked AX had reduced monomeric ferulic acid content in comparison with the other samples indicating oxidative ferulic acid dimerization. The bile acid-binding capacity of crosslinked AX was significantly higher than that of the non-treated, or hydroxyl radical-oxidized samples suggesting that the gel structure enhanced the bile acid retention capacity of the polymer. This result suggests that crosslinked AX may have enhanced cholesterol lowering effect compared with native AX. Furthermore, it was demonstrated that hydroxyl radical oxidation of AX lowered the water absorption of white flour blended with AX, and reduced the viscosity measured with Rapid Visco Analyser while crosslinked AX increased these values. The modified functionality might open up new prospects of the application of oxidized AX in functional food.

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1. Introduction

There is growing evidence that sufficient daily intake of cereal fibres is protective against wide-spread, diet-related diseases (Fardet, 2010). Feruloylated arabinoxylan (AX) is one of the most important cereal fibre components (Henry, 1985) and is supposed to possess health-promoting effects (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2011), which makes AX a promising ingredient in value-added, cereal-based food production (Mendis & Simsek, 2014). However, AX incorporation alters the technological properties (Döring, Nuber, Stukenborg, Jekle, & Becker, 2014) and often causes undesirable product quality (Courtin & Delcour, 2002; Krishnarau & Hoseney, 1994), presumably due to viscosity-related physical and ferulic acid mediated chemical interactions (Noort, van Haaster, Hemery, Schols, & Hamer, 2010; M.; Wang, van Vliet, & Hamer, 2004).

Several researchers investigated the modification of AX in order to eliminate the negative effects of AX on food quality. A common approach is the enzymatic degradation of AX with endoxylanases to improve dough functionality and bread quality. Endoxylanases solubilise water-unextractable AX contributing to increased dough stability, fermentation stability, resistance to mechanical stress, and oven rise (Courtin & Delcour, 2002). Another well-studied approach for AX modification is the oxidative crosslinking of AX through phenoxy radical-mediated oxidative dimerization of feruloyl units (Niño-Medina et al., 2009; Stone & Morell, 2009). Crosslinking of AX has been shown to affect the water absorption (WA) of rye dough (Buksa et al., 2014) and rye bread quality (Buksa, Nowotna, & Ziobro, 2016) when used as flour additive. AX oxidation is also possible with hydroxyl radical treatment. Hydroxyl radicals do not provoke gel formation, but a decrease in the viscosity of aqueous AX solutions, which suggests a decrease in molecular size (Bagdi, Tömöközi, & Nyström, 2016). Hydroxyl radical-mediated modification was shown to modify the swelling power and to improve the bile acid-binding capacity of cereal beta-glucan (de

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Moura, Pereira, da Silva, Zavareze, da Silveira Moreira, Helbig, & Dias, 2011), but similar studies on AX are not yet available. Bile acid-binding (the restriction of bile salt re-absorption) by dietary fibre components is believed to be one of the key mechanisms in the cholesterol-lowering behaviour of dietary fibres, although the exact mechanism of action is not yet understood (Gunness & Gidley, 2010).

The objective of the present study was the investigation of the effect of hydroxyl radical oxidation and oxidative crosslinking (peroxidase/hydrogen peroxide) on structural and functional properties of AX. Structural examinations cover ferulic acid content, arabinose/xylose ratio, and molecular size distribution. Bile acid-binding was examined with kinetic analysis across a dialysis membrane; technological properties (dough forming capability and pasting properties) were investigated with a micro-doughLAB instrument and with a Rapid Visco Analyser in a 4 g/100 g AX containing flour model system.

2. Materials and methods

2.1. Chemicals and materials

AX preparation was produced from aleurone-rich flour as described earlier (Bagdi et al., 2016). 10 mg of peroxidase (from horseradish, 150 U/mg, Sigma–Aldrich, Buchs, Switzerland) enzyme preparation was dissolved in 5 ml of 50 mM sodium phosphate buffer (pH 6.0).

Hydrogen peroxide (30 wt % in H_2O), iron (II) sulphate heptahydrate, D-(+)-xylose, L-(+)-arabinose, ferulic acid, *o*-coumaric acid, sodium cholate, sodium deoxycholate, sodium glycocholate, sodium taurocholate, acetic acid, sodium acetate, acetonitrile, and hydrochloric acid were originated from Sigma–Aldrich (Buchs, Switzerland). Ascorbic acid and absolute ethanol were purchased from Fluka (Buchs, Switzerland). Sodium hydroxide was obtained from Fischer Chemicals AG (Zürich, Switzerland). Ultrapure water was used for all of the experiments (Merck Millipore, Merck KGaA, Darmstadt, Germany.).

2.2. Preparation of the oxidized AX samples

AX solution was prepared by dispersing the AX preparation in water. The dispersion was incubated in a water bath at 80 °C with continuous stirring, until the solid material was entirely dissolved. The solution was transferred into a screw-cap bottle and oxidizing agents were added: Non-oxidized AX (NOX): 0.02 mg/ml AX in water; hydroxyl radical oxidized 1 and 2 (·OH1 and ·OH2): 0.02 mg/ml AX, 50 μM Fe, 500 μM ascorbic acid, and H_2O_2; AX crosslinked with peroxidase (POD); 20 μM H_2O_2, 30 U/ml peroxidase enzyme. The samples were incubated in a water bath at given temperature for 180 min: NOX, ·OH1, and POD at 20 °C, ·OH2 at 80 °C, producing samples at two different levels of hydroxyl radical oxidation (·OH1<·OH2). These parameters were chosen based on the results of our previously published work (Bagdi et al., 2016) to produce differently oxidized samples. At the end of the oxidation treatment the viscosity of the hydroxyl radical treated solutions and the gelling behaviour of the crosslinked material were measured as described earlier (Bagdi et al., 2016). The viscosity values (NOX: 0.16 Pa s (100%), ·OH1: 0.07 Pa s (42%), ·OH2: 0.03 Pa s (21%)) and the gelling behaviour of POD were in accordance with our earlier results (Bagdi et al., 2016), and showed that samples oxidized to different extents were produced. Afterwards, equal amount of ethanol was added to the samples to precipitate the polymer. The samples were stirred vigorously and transferred into ultracentrifuge tubes. Ultracentrifugation was carried out at 48000 rcf for 40 min. Afterwards, the supernatant was discarded and the pellet was washed 3 times with 1:1 EtOH:H_2O mixture in order to rinse off the oxidizing agents, and once with EtOH to inactivate the enzyme. The pellet was freeze-dried and pulverized with a ball mill equipped with a 15 ml grinding bowl and one grinding ball with a diameter of 15 mm (Mini-Mill Pulverisette 23, Fritsch, Idar-Oberstein, Germany). The oxidized AX samples were stored in glass containers at room temperature.

2.3. Ferulic acid and arabinoxylan content measurements

Ferulic acid content was determined according to Rouau et al. (2003), using *o*-coumaric acid as internal standard with high performance liquid chromatograph (HPLC, Agilent 1100, Switzerland) equipped with an xBridge Shield RP18 3.5 μm (3.0 × 150 mm). Measurements were carried out in triplicate.

Arabinoxylan content and the degree of arabinoxylan branching (arabinoxylan/xylose ratio) were determined with a GC-FID method (Gebruers, Courtin, & Delcour, 2009), using a Perkin Elmer Clarus 500 gas chromatograph (Perkin Elmer Inc., Norwalk, Connecticut, USA) equipped with an Elite 17 column (60 m × 0,25 mm × 0,25 μm). Arabinose and xylose content was determined as alditol acetates following reduction, acid hydrolysis and acetylation of the samples. AX content was calculated as 0.88 × (g/100 g arabinose + g/100 g xylose). The measurements were carried out in triplicate.

2.4. Determination of molecular size with size-exclusion chromatography

A chromatography method was carried our based on Loosveld & Delcour, 2000. Aliquots (2.0 mg) of the oxidized AX samples were solubilised in 3 g/l NaCl solution at 80 °C and the solution was filtered (0.45 μm). Afterwards the samples were examined on a Shodex, OHpak 10 μm SB-804 HQ 200 A (300 mm × 8 mm) gel filtration column (Phenomenex Inc, Torrance, California, USA) by elution with 3 g/l NaCl (0.3 ml/min at 38 °C). The refractive index of the eluate was monitored using a Flexar RI LC detector (PerkinElmer Inc) at 38 °C. Molecular weight (MW) markers were Shodex Standard P-82 pullulans with an MW range of 5.900–708.000. The measurements were carried out in triplicates.

2.5. Passage kinetics of bile salts

The bile salt retention capability of AX was determined with *kinetic analysis across a dialysis membrane* according to Gunness, Flanagan, Shelat, Gilbert, and Gidley (2012). Phosphate buffer (0.1 M, pH 6.2) was used throughout the analysis. Bile salt mixture (40 mM) that consisted of 10 mM sodium cholate, 10 mM sodium deoxycholate, 10 mM sodium glycocholate, and 10 mM sodium taurocholate was prepared using the phosphate buffer. AX sample (NOX, ·OH1, ·OH2, and POD) were dissolved at 80 °C in the phosphate buffer. To investigate the barrier properties, AX solution and the bile salt mixture were transferred into a dialysis device (slide-A-Lyzer MINI Dialysis Device, 10K MWCO, 2 mL; Thermo Scientific Pierce Protein Research Products, Rockford, Illinois, United States) to reach a final concentration of 0.02 g/ml and 10 mM respectively. Micelle formation was detected in the mixture with light scattering measurements (data not shown), showing that the applied concentration was higher than the critical micellar concentration, which is desirable for bile acid-binding tests (Gunness & Gidley, 2010). The blank was prepared by replacing AX solution with the phosphate buffer. The dialysis device was placed into a 50 ml-conical tube that was previously filled with 44.5 ml buffer. The tube was sealed with a screw-top cap, and was incubated under shaking (280 rpm and 37 °C). After 2, 4, 8, 12, 16, 24, and 48 h of incubation

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