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Effect of water-extractable arabinoxylans from wheat aleurone and bran on lipid peroxidation and factors influencing their antioxidant capacity

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ABSTRACT

Dietary lipid hydroperoxides (LOOH) are implicated in the rise of colon cancers. We investigated the effect of water-extractable arabinoxylans (WEAX) on lipid peroxidation under simulated gastric conditions. Water-extractable fractions containing mostly arabinoxylans were isolated from wheat aleurone and wheat bran and fractionated by stepwise precipitation with $(NH_4)_2SO_4$ at 50% and 75% saturation. LOOH concentration (121.2 ± 3.8 μM/g) of grilled chicken breast muscle increased by 30%; however, it remained unaltered or decreased (≤35%) depending on type and concentration of WEAX fractions. Antioxidant capacity of WEAX fractions based on DPPH, ABTS and ORAC was 28.2 – 147.9, 91.2 – 355.3, and 185.9 – 527.5 μM TE/g, respectively. The content of ferulic acid residues in WEAX fractions (R = 0.99) and relative proportions of monosubstituted xylose residues (R = 0.80) influenced the antioxidant capacity. Consumption of diets rich in feruloylated WEAX may offer protection against oxidative damage in the gastrointestinal tract.

1. Introduction

Numerous studies have shown that consumption of fiber from cereal grains is associated with reduced risk of cancers of colon, however, the underlying mechanism of this association has not been fully elucidated (Aune et al., 2011; Zeng, Lazarova, & Bordonaro, 2014). The dietary fiber constituents may exert anticancer effects by proliferating healthy gut microbiota, providing substrate for increasing short chain fatty acids production, increasing fecal bulk and viscosity, and/or binding of potential cancer-causing agents (Zeng et al., 2014). Studies have linked consumption of high fat diets with increasing incidences of colorectal and colon cancers (CRC) (Larsson, Bergkvist, & Wolk, 2005). In general, animal fats, especially from red meat, present a higher risk compared to lipids from plant sources. Indeed, a meta-analysis study found that high consumption of red meat increases the risk of colorectal and colon cancers (Larsson & Wolk, 2006). Haemoglobin or myoglobin is known to promote lipid peroxidation (Kanner & Lapidot, 2001). Both haemoglobin and lipid hydroperoxides (LOOH) are capable of inducing DNA oxidation at high concentration (> 100 μM) (Angeli et al., 2011). Synergistic effects of haemoglobin and lipid hydroperoxides on genotoxicity have also been reported (Angeli et al., 2011; Kanner & Lapidot, 2001). Heme-iron facilitates degradation of LOOH (Carlsen, Møller, & Skibsted, 2005) to peroxyl and alkoxyl radicals, which may eventually damage the DNA. Thus presence of antioxidants may mitigate the development or propagation of CRC associated with high fat or red meat diets. Recently, reports have shown that feruloylated arabinoxylan, a major constituent of cereal dietary fiber, possesses antioxidant properties (Rao & Muralikrishna, 2006).

Arabinoxylans (AX) are predominant constituents of dietary fiber in cereal grains (60–70%) (Antoine et al., 2003); however, their exact content and molecular structure vary with source and/or grain tissue. Whole grain wheat contains about 1.3 – 2.7% AX of which 14–30% is water extractable (Gebruers et al., 2008). Wheat bran contains 8–18% AX of which 2.2 – 5.5% are water extractable (Gebruers et al., 2008). AX content for hand-isolated wheat aleurone and pericarp was 20% and 45%, respectively (Antoine et al., 2003). Arabinoxylans consist of a linear chain backbone of (1→4) linked β-D-xylopyranosyl (Xyl*p*) residues with α-L-arabinofuranosyl (Ara*f*) residues linked to the xylan backbone. Ara*f* residues are attached to some of the Xyl*p* residues at C(O)–2, C(O)–3 and/or at both C(O)–2,3 positions (Izydorczyk & Biliaderis, 1995). Arabinoxylans from the secondary walls in the pericarp and testa tissues of wheat may carry α-D-glucuronic acid residues (or its 4-O-methyl ether) (Ma et al., 2012). A distinct feature of cereal arabinoxylans is the presence of hydroxycinnammic acid residues, mostly ferulic and some p-coumaric, esterified to C(O)–5 of Ara*f* that are linked to the Xyl*p* residues at position C(O)–3

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(Smith & Hartley, 1983). The ratio of arabinose to xylose, pattern of arabinose substitution, degree of feruloylation, and molecular weight of AX vary greatly among and within cereal grains (Izydorczyk & Biliaderis, 1995).

The presence of phenolic moieties in arabinoxylan oligosaccharides has been linked to their antioxidant and antimicrobial potential (Katapodis et al., 2003; Yuan, Wang, & Yao, 2005). Several studies showed that ferulic acid in bran have strong anti-inflammatory properties, inhibit chemically induced carcinogenesis in rats and plays a role in inhibiting lipid peroxidation and low density lipoprotein (LDL) oxidation, and scavenging oxygen radicals (Rondini et al., 2004). However, in recent studies on corn arabinoxylans, Ayala-Soto, Serna-Saldívar, García-Lara, and Pérez-Carrillo (2014) showed that the antioxidant capacity of AX extracted from corn was not influenced by the content of ferulic acid residues associated with these polymers. The antioxidant properties of AX may also be influenced by complex and diverse molecular structure of these polysaccharides associated with their cellular origin. The objective of this study was, therefore, to investigate the role of structurally variable arabinoxylans obtained by water extraction from wheat bran and wheat aleurone in preventing lipid peroxidation under gastric conditions.

2. Materials and methods

2.1. Sample and chemicals

A commercial wheat aleurone (Grainwise wheat aleurone) was a gift from Cargill Limited and Horizon Milling (Wichita, Kansas, U.S.A.). It constitutes 4.5%, 15.2%, 7.4%, and 2.5% lipid, protein, ash, and starch, respectively. Hard red winter wheat bran was purchased locally from Bulk Barn (Winnipeg, Manitoba, Canada). Its moisture, ash and protein content were analysed to be 5.8%, 5.3%, and 11.1%, respectively. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchoman-2-carboxylic acid (trolox), porcine pancreas α-amylase (EC 3.2.1.1, Type I-A), ferulic acid standard, sugar standards (D-glucose, D-xylose, D-arabinose, D- mannose, D-glucouronic acid and D-galactose) and dialysis tubing cellulose (molecular weight cut off (MWCO) 12000), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$), 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), fluorescein, and fuller's earth were bought from Sigma-Aldrich (Milwaukee, WI, USA). Ammonium sulphate, all acids and organic solvents were bought from Fischer Scientific (Whitby, Ontario, Canada). All chemicals used were of analytical or HPLC grade.

2.2. Preparation of water extractable arabinoxylan fractions from wheat bran and aleurone

Wheat bran and wheat aleurone samples (~200g) were boiled under reflux in 2 L aqueous ethanol (80%, v/v) at 85 °C for 2 h to inactivate endogenous enzymes. Samples were cooled to room temperature and centrifuged (10 000g, 4 °C and 20 min). The residue was washed twice with 500 mL 95% ethanol and the pooled supernatants were discarded. The residue was air dried in a fume-hood overnight at room temperature.

Water extractable fractions were isolated at 45 °C according to the method described by Izydorczyk and Biliaderis (1994) with some modifications. The air dried bran or aleurone (150g) were mixed with 1.5 L deionised water. The mixture was stirred overnight and centrifuged (12000*g*, 4 °C) for 20 min. The residue was washed twice with deionized water and the supernatant pooled together. The supernatant was boiled at 95 °C for 15 min to denature soluble proteins. After centrifugation, the supernatant was mixed with acid washed celite (10g/L) for 20 min to remove proteins followed by centrifugation. Residual proteins were further removed by mixing the supernatant with Fuller's earth (20g/L) for 20 min. The mixture (celite or Fuller's earth) was stirred slowly using a magnetic stirrer at room temperature to facilitate protein adsorption. The mixture was then centrifuged (12000*g*, 4 °C) for 20 min. The supernatants were mixed overnight with α-amylase (1821 U/L) to remove starch. The sample was heated to 95 °C and further purified using celite and Fuller's earth as before. The purified material was fractionated by graded ammonium sulphate (AS) precipitation. Three fractions were obtained at 50%, 75% and 100% AS saturation. The sample was finally dialysed (12 kDa cut-off membrane) for 48 h and freeze dried. The material collected at 100% AS saturation was discarded as it did not contain significant amount of AX. The materials collected from wheat aleurone were labelled (WA) followed by the concentration of AS at which they were obtained (WA-f_{50} and WA-f_{75}). Similarly, the extracted materials from wheat bran (WB) were denoted as WB-f_{50} and WB-f_{75}.

2.3. Characterisation of the water extractable arabinoxylan extracts

Protein content (% N X 6.25) was determined by method 46-30.01 (AACC International 1999). Monosaccharide composition was determined by gas liquid chromatography as described by Izydorczyk et al. (2014). Molecular weight of isolated WEAX fractions was determined using a high-performance size-exclusion chromatography system using a method described by Irakli, Biliaderis, Izydorczyk, and Papadoyannis (2004). The samples were dissolved in $NaNO_3$ buffer (1.5 mg/mL). A Shodex column (806, 804) and $NaNO_3$ buffer were used at 30 °C. ^1H NMR spectroscopy was performed on a Bruker Avance 500 III HD spectrometer operated at 500.13 MHz and 20 °C. Samples (5 mg) were dissolved in 1 mL D_2O.

2.4. Prevention of lipid hydroperoxides formation in simulated gastric digestion

A Kanner and Lapidot (2001) method for generation of lipid peroxide in the stomach was used with modifications. WEAX fractions were dissolved in simulated gastric fluid (SGF) (5.52 mM KCl, 0.72 mM KH_2PO_4, 20 mM $NaHCO_3$, 37.76 mM NaCl, 0.08 mM $MgCl_2(H_2O)_6$, 0.4 mM $(NH_4)_2CO_3$ and 4000 U/mL porcine pepsin). Four concentrations (0, 0.25, 0.5 and 0.75 mg/mL) of WEAX fractions were used. Grilled lean chicken breast (30g) was blended in 100 mL SGF (ice cold) using a kitchen blender at maximum speed for 2 min. Meat suspension (20 mL) was transferred into four conical flasks with a screw cap (50 mL). The suspension was incubated in a shaking water bath for up to 2 h at 37.0 °C and 250 rpm. Samples were drawn at 0, 40, 80 and 120 min for determination of lipid hydroperoxides. Lipid hydroperoxides were determined by FOX2 assay with some modifications. Samples (1 mL) (6 times) were transferred in 30 mL test tubes covered in an aluminum foil. Triphenylphosphine (TPP) (1 mL) and 0.5 mL acidified methanol was added to 3 of the 6 test tubes. To the remaining test tubes, 1 mL of methanol was added instead of TPP. The mixture was incubated at room temperature with periodic mixing (votex) for 30 min. FOX 2 reagent (22.5 mL) was added followed by incubation for 30 min at room temperature and darkness. It was later centrifuged for 10 min at 15000 × *g* and 25 °C. The supernatant (2 mL) was used for absorbance measurement (Ultrospec 1100 Pro, UV/Visible Spectrophotometer (Biomicron Ltd, Cambridge, CB4QFJ, England)) at 560 nm. The experiment was repeated 3 times for each sample.

2.5. Determination of antioxidant activity using DPPH, ORAC, and ABTS assays

Antioxidant capacity assays for DPPH, $ABTS^+$ and ORAC were performed according as described in Malunga and Beta (2015a).

Effect of ferulic acid on the antioxidant activity of WEAX fractions was done using two approaches. In the first approach, samples were saponified in 2 M NaOH under nitrogen at 4 °C. After 4 h, samples were neutralized using 6 M HCl. Ethanol (4 volumes) was added to precipitate AXs. After centrifugation, the residue was washed twice with