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Antioxidant activities of corn fiber and wheat bran and derived extracts

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ABSTRACT

The antioxidant activity of ground untreated corn fiber and wheat bran against lipid oxidation of rapeseed-fish oil-in-water emulsions was determined with respect to the formation of primary (lipid hydroperoxides) and secondary (propanal and hexanal) oxidation products. Corn fiber inhibited the formation of lipid hydroperoxides secondary products effectively at a concentration of 800 mg/kg emulsion, but wheat bran showed only low antioxidant effects. It was shown that the activity was based on the action of bound hydroxycinnamates like ferulic acid and p-coumaric acid, which was proven by the application of ferulated oligosaccharides (FOm) that showed higher activity than free ferulic acid on a molar basis. Enzymatic treatment with ferulic acid esterases did not increase the antioxidative activity. Extraction of corn fiber and wheat bran with methanol and isopropanol resulted in products which contained only low amounts of free soluble hydroxycinnamates and their marked activity against lipid oxidation and in antioxidant tests (TEAC, DPPH) gave reason for further fractionation. It has been shown that the polyaminconjugates diferuloylputrescine and p-coumaroyl-feruloylputrescine, which are major compounds of the corn fiber methanolic extract, exhibited high antioxidant activity and it can be assumed that they will take part in the antioxidant action of untreated fibers.

1. Introduction

Corn fiber and wheat bran are agro-industrial derived by-products, which arise during starch and flour production and are associated with high dietary fiber content. Cell walls of monocotyledons consist mainly of cellulose, hemicelluloses and pectin. It is well known that they contain numerous hydroxycinnamic acids mainly covalently bound to polysaccharides via ester linkages (Gallardo, Jimenez, & Garcia-Conesa, 2006). Ferulic acid is the most abundant hydroxycinnamic acid in cereals which is associated with the outer layers of the kernels (Andreasen, Christensen, Meyer, & Hansen, 2000; Barron, Surget, & Rouau, 2007). Other hydroxycinnamic acids were found in smaller quantities (p-coumaric acid, sinapic acid, caffeic acid) (Rhodes, Sadek, & Stone, 2002; Run Cang, Xiao Feng, & Shi Hong, 2001; Yadav, Moreau, & Hicks, 2007). Diferulic acids are products of the oxidative coupling of ferulic acid and they may form crosslinks in the arabinoxylan network and exert an important role in the stability of non-lignified cell walls (Bunzel, Ralph, Marita, Hatfield, & Steinhart, 2001; Saulnier & Thibault, 1999). The industrial application of hydroxycinnamates had raised interest because they and their conjugates were shown to be bioactive compounds possessing beneficial antioxidant activities and health benefits (Thiyam, Stöckmann, & Schwarz, 2006; Faulds, 2010).

Ferulic acid acts as a radical scavenger and displays antioxidant activity in lipid containing foods (Oehlke, Heins, Stöckmann, & Schwarz, 2010). The antioxidative capacity of ferulic acid is based on its ability to easily abstract a hydrogen atom to form a resonance stabilized phenoxyl radical which is unable to initiate or propagate a radical chain reaction (Graf, 1992). The increase of health beneficial polyunsaturated fatty acids in foods requires improved protection from lipid oxidation as the vulnerability increases with the degree of unsaturation. As a result, interest in finding natural sources of antioxidants has been raised, but commonly used natural antioxidants like herb extracts often possess strong flavors (Frankel, Huang, Aeschbach, & Prior, 1996). Wheat bran and corn fiber do not display strong tastes of their own and are therefore an interesting alternative source of natural antioxidants.

There are two different procedures described to retrieve phenolic acids from fiber. On the one hand, alkaline hydrolysis of plant material is often applied to determine the content of cell wall bound phenolics. Further purification steps, such as solvent extraction, are necessary to obtain a product that could be used as

Abbreviations: Cf, corn fiber; CFP, p-coumaroyl-feruloylputrescine; DFP, diferuloylputrescine; FA, ferulic acid monomer; FOm, ferulated oligosaccharides; RFO, rapeseed fish oil mixture; Tx, trolox; Wb, wheat bran.

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food additive (Tilay, Bule, Kishenkumar, & Annapure, 2008). On the other hand, another method to release cell wall bound phenolics is enzymatic hydrolysis of cell walls with cell wall degrading enzyme complexes. Ferulic acid and other hydroxycinnamates were successfully released from corn fiber and wheat bran by ferulic acid esterases from *Aspergillus niger* and *Humicola insolens* (Benoit et al., 2006; Faulds, Mandalari, LoCurto, Bisignano, & Waldron, 2004).

Extracts derived from corn fiber and wheat bran containing free hydroxycinnamic acids, showed significant antioxidative properties in vitro such as scavenging of 2,2′-azinobis 3-ethylbenzothiaz oline-6-sulfonic acid (ABTS) cation, 2,2-diphenyl-1-picryhydrazyl (DPPH) or chelating activities against Fe^{2+} and Cu^{2+}, wherefore the contribution of bound phenolics to the predicted antioxidant activity of whole brans is often underestimated (Andjelkovic et al., 2006; Liyana-Pathirana & Shahidi, 2006; Lopez-Martinez et al., 2009).

The aim of the study is the investigation of the antioxidant effect of untreated and enzymatically treated corn fiber and wheat bran on lipid oxidation in systems containing fish oil. The action of untreated fibers, cell wall degrading enzymes and the influence of different extraction procedures with organic solvents were investigated to estimate the antioxidative action of bound or free ferulic acid in a lipid oxidation model.

2. Materials and methods

2.1. Materials and chemicals

Samples of corn fiber were kindly provided by Habema GmbH, Hamburg, Germany. Wheat bran was purchased on the local market. Refined fish oil was provided by Cognis Deutschland GmbH & Co. KG, Illertissen, Germany and contained in sum approximately 267 mg/g eicosapentaenoic acid and docosahexaenoic acid as specified by the manufacturer. Refined rapeseed oil was purchased on the local market and contained 290 mg/g polyunsaturated fatty acids. The enzymes Depol 740 L and Depol 670 L were purchased from Biocatalysts Limited, Wales, UK. Aluminum oxide, Amberlite XAD-2, ammonium thiocyanate, α-amylase (Termamyl®), Brij 58®, p-coumaric acid, ferulic acid, horseradish peroxidase, propyl gallate and Trolox were purchased from Sigma Aldrich, Munich, Germany. Acetic acid, acetone, barium chloride dihydrate, hydrochloric acid, ethyl acetate, n-heptane, n-hexane, iron (III) chloride dihydrate, iron (II) sulfate heptahydrate, isopropyl alcohol, methanol, sodium acetate, sodium hydroxide, sodium sulfate and trifluoracetic acid were purchased from Carl Roth GmbH & Co. KG, Karlsruhe, Germany.

2.2. Preparation of extracts

All fibers were ground in a ball mill, sieved (<125 μm) and stored in a desiccator prior to analysis. Corn fiber (Cf) was treated with Depol 740 L which is a mono-active feruloyl esterase from *H. insolens* with a ferulic acid esterase activity of 36 U/g (manufacturers' datasheet). Two grams of corn fiber were incubated for 24 h in an incubation shaker with 2.5 ml of enzyme in acetate buffer at pH 5 at a final volume of 100 ml. Wheat bran (Wb) was incubated under same conditions but with Depol 670 L, a cell wall degrading enzyme mixture with feruloyl esterase activity. Resulting hydrolysates were separated in three different extraction products which were further prepared. One aliquot was freeze dried and resulted in a powdered product containing the aqueous supernatant and non-hydrolyzed fibers (Cf-T; Wb-T). Another aliquot was centrifuged and freeze dried (Cf-S; Wb-S) containing only the supernatant. The third aliquot was acidified (HCl; pH <2) and the supernatant was extracted three times with EtOAc. The combined organic layers were dried over sodium sulfate and brought to dryness by a rotary evaporator (Cf-EtOAc, Wb-EtOAc).

For preparation of non-enzymatic extracts two grams of fibers were weighted into centrifugation tubes and diluted with 30 ml of methanol or isopropyl alcohol and left to stand overnight at room temperature and further sonificated (cycle 9, 90% power, Bandelin sonopuls, sonication probe VS 70, Berlin, Germany) for 2 min. After centrifugation at 9000 rpm for 10 min the supernatant was decanted, 20 ml solvent was added again and extraction steps were repeated two times. The combined supernatants were dried on a rotary evaporator and stored at a freezer at −20 °C until analysis (Cf-MeOH, Cf-P; Wb-MeOH, Wb-P).

Ferulated oligosaccharides (FOm) from corn fibers were produced by mild acid hydrolysis according to the method of Saulnier, Vigouroux, and Thibault (1995) as described earlier (Bauer, Harbaum-Piayda, & Schwarz, 2012). Therefore destarched fibers were applied to acid hydrolysis with 50 mmol trifluoracetic acid and ferulated oligosaccharides were gained after column chromatography on Amberlite XAD-2. Two different ferulated fragments of the arabinoxylan network were identified tentatively as feruloyl arabinose (FA) and feruloyl-arabinose-xylose ester by HPLC–ESI-MS (Bauer et al., 2012).

2.3. Fractionation of extracts

The methanolic corn fiber extract was fractionated by preparative HPLC (Agilent 1100 series) using a 250 mm × 21 mm, 5 μm RP C18ec Nucleodur column (Macherey-Nagel). Eluents were 100% water (A) and 100% methanol (B). The gradient was developed at initial conditions 30% B which rose up to 100% B within 9 min and kept on 100% B for another 7 min at a flow rate of 20 ml/min. Four fractions were separated as follows: fr. 1, 2.5–4.3 min; fr. 2, 4.3–7.7 min; fr. 3, 7.7–10.3 min; fr. 4, 10.1–14 min.

2.4. Alkali treatment

The amounts of bound Hydroperoxides and their coupling products in untreated corn fiber and wheat bran were determined after alkaline hydrolysis as described by Kroon, Garcia-Conesa, Fillingham, Hazlewood, and Williamson (1999). 2 g of sample were hydrolyzed for 24 h with 60 ml of 2 mol/L NaOH under hydrogen atmosphere. The supernatant was obtained after centrifugation at 9000 rpm for 10 min. An aliquot of 5 ml was adjusted to pH <2 by the addition of hydrochloric acid and extracted three times with ethyl acetate. Combined organic layers were pooled and dried over sodium sulfate. The solvent was removed by rotary evaporation at 40 °C and the residues were redissolved in 10 ml methanol and used for HPLC analysis.

2.5. HPLC analyses

For quantitative analysis of phenolic acids a HP 1100 HPLC (Agilent Technologies, Waldbronn, Germany) equipped with a diode array detector was used as described by Bauer et al. (2012). Ferulic acid, p-coumaric acid, and diferulic acids were quantified by external standard calibration. Diferuloylputrescine was isolated by preparative HPLC from the methanolic corn fiber extract and used as reference compound for the quantification of diferuloylputrescine and p-coumaroyl-feruloylputrescine. The sum of these compounds was expressed as ferulic acid equivalents based on their molarities.

2.6. Emulsion preparation and storage

Oxidation experiments were carried out with 10% oil in water emulsions with 1% Brij 58 as emulsifier. The aqueous phase consisted of 0.2 mol/L acetic acid/Na-acetate pH 5.0 buffer solution. An oil mixture of rapeseed oil and refined fish oil (RFO) 70:30 (w/w)