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Inhibitory effects of wheat bran extracts on human LDL oxidation and free radicals

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Abstract

Bran extracts of Akron and Trego wheat grown at three locations in Colorado were examined for their inhibitory activities against lipid peroxidation in human low-density lipoprotein (LDL), oxygen radical absorbance capacity (ORAC), and radical scavenging properties against stable 2,2-diphenyl-1-picryhydrazyl radical ($DPPH^{\bullet}$), peroxide anion radical $O_2^{\bullet -}$, and radical cation 2,2′-azinobis-(3-ethylbenzothiazoline sulfonate) ($ABTS^{\bullet +}$). All bran extracts significantly reduced lipid peroxidation in LDL. The greatest activity in suppressing LDL oxidation was detected in Akron bran from Fort Collins (1.56 mg TBARS reduction/g bran), while the Trego bran from Fort Collins showed the lowest activity (1.03 mg TBARS reduction/g bran). The Trego bran from Fort Collins had the highest ORAC of 18.5 μmole TE/g bran, followed by Trego bran (Walsh), Akron bran (Walsh), Akron bran (Fort Collins), Akron bran (Julesburg), and Trego bran (Julesburg). Bran extracts reacted with and quenched 49–72% $DPPH^{\bullet}$ and 21–37% $O_2^{\bullet -}$ in the reaction mixtures under the experimental conditions. The $ABTS^{\bullet +}$ scavenging capacity of the six bran extracts was in the range of 5.19–8.12 μmole Trolox Equivalent/g bran, with the Trego bran from Walsh showing the greatest scavenging activity. Ferulic acid was the predominant phenolic acid present in both Akron and Trego bran, along with syringic, *p*-hydroxybenzoic, vanillic, and coumaric acids. No correlation was inferred among individual antioxidant activities under the experimental conditions. The data suggest that wheat bran antioxidants may have the potential application in preventing the early development of atherosclerosis and other health problems associated with radical mediated damages.

Keywords: Wheat; Bran; Radical scavenging; Antioxidant; Ldl; Radical anion; DPPH; Peroxide anion; Oxygen radical

1. Introduction

Low-density lipoprotein (LDL) oxidation by different mechanisms, including free radical mediated oxidative reactions in both lipid and protein fractions, is considered a pivotal step in the early development of atherosclerosis (Pearson, Frankel, Aeschbach, & German, 1997). It has been widely accepted that antioxidants may inhibit LDL oxidation, and eventually may reduce the risk of cardiovascular diseases (Meyer, Yi, Pearson, Waterhouse, & Frankel, 1997; Pearson et al., 1997; Denicola, Batthyany, Lissi, Freeman, Rubbo, & Radi, 2002; Rao 2002; Filipe et al., 2002). Antioxidant activities have been detected in a number of wheat varieties and wheat-based food products (Onyeneho & Hettiarachchy, 1992; Baublis, Lu, Clydesdale, & Decker, 2000; Zielinski & Kozlowska, 2000; Yu, Haley, Perret, Harris, Wilson, & Qian, 2002a, Yu, Haley, Perret, & Harris, 2002b, Yu, Perret, Davy, Wilson, & Melby, 2002c; Yu, Scanlin, Wilson, & Schemidt, 2002d). These reported antioxidant activities included the radical scavenging capacity against the stable 2,2-diphenyl-1-picryhydrazyl radicals ($DPPH^{\bullet}$) and 2,2′-azino-di[3-ethylbenzothiazoline sulfonate] radical cation ($ABTS^{\bullet +}$) generated by an enzymatic method, suppressing lipid peroxidation in oils, inhibiting liposome lipid peroxidation induced by both radical and iron/ascorbic acid, and chelating activity against Fe^{2+}. These previous studies also showed that antioxidative properties might

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vary among wheat cultivars (Zielinski & Kozlowska, 2000; Yu et al., 2002a; Yu, Perret, Harries, Wilson, & Haley, 2003). Therefore, it is possible that wheat antioxidants may inhibit human LDL oxidation and prevent atherosclerosis development.

Oxygen radical absorbance capacity (ORAC) measures the protective potential of a selected antioxidant on protein oxidation mediated by peroxyl radicals (Ou, Hampsch-Woodill, & Prior, 2001). The peroxyl radicals are induced by 2,2′-azobis(2-amidinopropane) dihydrochlorides (AAPH). ORAC value reflects the ability of an antioxidant in suppressing the formation of peroxyl radicals and in terminating the radical attack on protein molecules. To our knowledge, no ORAC value was reported for wheat or wheat-based food products produced in the United States, although significant ORAC values of Swiss red wheat and fractions were detected in our recent studies (Zhou, Laux, & Yu, 2004).

Phenolic compounds are commonly produced in plants including wheat grain. Phenolic acids in wheat grain, including ferulic, vanillic, p-coumaric, caffeic, and chlorogenic acids, are concentrated in the bran portion of cereal kernels (Onyeneho & Hettiarachchy, 1992). These compounds may contribute to the total antioxidant activities of wheat (Onyeneho & Hettiarachchy, 1992). Our previous studies of antioxidants in Swiss red wheat also showed that antioxidant compounds are mainly distributed in the bran fractions (Zhou et al., 2004), suggesting that bran may be a good dietary source of wheat antioxidants. This study was conducted to evaluate bran extracts of selected wheat varieties grown in Colorado (US) for their potential inhibitory effects on human LDL oxidation, oxygen radical absorbance capacities (ORAC), and free radical scavenging activities against $DPPH^{\bullet}$, peroxide anion radical $O_2^{\bullet -}$, and radical cation $ABTS^{\bullet +}$ generated by a chemical method. The correlation between the inhibition of LDL oxidation and each of the tested other antioxidant properties was also examined.

2. Materials and methods

2.1. Materials

Hard winter wheat varieties of 'Akron' and 'Trego' (*Triticum aestivum* L.) were used for this study. Wheat grains were obtained from two nonirrigated field locations in eastern Colorado (Julesburg and Walsh) and one irrigated field location along the Front Range of Colorado (Fort Collins). Low-density lipoprotein from human plasma, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, dimethyl sulfoxide, lauryl sulfate sodium salt, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (St. Louis, MO), while 2,2′-azobis (2-amino-propane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA (Richmond, VA). β-cyclodextrin was purchased from Cyclolab R & D Ltd. (Budapest, Hungary). All other chemicals and solvents were of the highest commercial grade and used without further purification.

2.2. Preparation of bran extracts

Wheat grain from each location was milled on a Brabender Quadromat Jr. experimental mill for separation into bran and flour fractions. Ten grams of each ground bran was extracted with 100 ml of absolute ethanol under nitrogen at 20 °C for 15 h (Yu et al., 2003). Ethanol was removed under vacuum from a known volume of the ethanol extract, and the solid residue was quantitatively re-dissolved in 1 ml of acetone and 9 ml of 70 g/l β-cyclodextrin prepared with 50% acetone. The resulting β-cyclodextrin-acetone solution was centrifuged to obtain a clear stock solution. In order to prepare dimethyl sulfoxide (DMSO) solution, the solid residue was quantitatively re-dissolved in DMSO. The stock solutions were kept in dark under nitrogen until further analysis.

2.3. Preparation of LDL

A commercial EDTA-containing solution of human LDL was dialyzed in a 100-fold volume of nitrogen gas saturated 0.01 mol/l phosphate buffer solution (PBS), pH 7.4, containing 0.16 mol/l NaCl (Ohta, Semboku, Kuchii, Egashira, & Sanada, 1997; Fernandes, Filipe, Freitas, & Manso, 1996; Esterbauer, Streigl, Puhl, & Rotheneder, 1989). The buffer was changed four times during 24 h. Protein content of the EDTA free LDL solution was determined by Bradford assay (Bradford, 1976), and a stock solution of 200 μg protein per ml was prepared with nitrogen saturated PBS for LDL oxidation assay, and stored under nitrogen at 4 °C in dark for no longer than 24 h.

2.4. Inhibition of LDL oxidation

Oxidation of LDL was initiated by the addition of a freshly prepared copper chloride solution in the EDTA-free LDL assay mixture containing SDS at a concentration of 10 g/l, and 20 μl of the DMSO solution of wheat antioxidants (Fernandes et al., 1996; Ohta et al., 1997). A control, containing no antioxidant, was performed using 20 μl of the DMSO. The final $CuCl_2$ concentration was 15 μmol/l, and final LDL content was 100 μg of protein per ml in all assay mixtures. The total volume of each assay mixture was 1.0 ml. The oxidation reaction was carried out at 20 °C for 60 min. The level of lipid oxidation in the assay mixture was then measured by