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Effects of region and cultivar on alkylresorcinols content and composition in wheat bran and their antioxidant activity

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ABSTRACT

This study evaluated the effects of cultivar and region on the composition of alkylresorcinols (ARs) of 24 wheat bran samples from 6 cultivars grown in four locations (Bath, Nairn, Palmerstone and Ridgetown) in Ontario (ON), Canada, using gas chromatography/mass spectrometry (GC/MS). Total phenolic content (TPC) of wheat bran extracts was determined by the Folin–Ciocalteau method and the antioxidant activity of wheat bran extracts was measured by 2, 2-Dipheny-1-picryhydrazyl radical (DPPH) scavenging activity and oxygen radical absorbance capacity (ORAC). The highest ARs content (μg/g) was found in cultivars Emmit (1522), Harvard (1305), Warthog (1170), and Superior (853), grown in Ridgetown. The relative saturated and unsaturated ARs (%) were 89 and 11, respectively. Total ARs content, their composition, TPC and antioxidant activity of wheat bran extracts were significantly ($P < 0.05$) affected by location and cultivar and their interaction. TPC, $\%DPPH_{dis}$, and ORAC values for different wheat bran sample extracts ranged from 3 to 58 (mg FAE/g), 5 to 68 (%), and 6 to 94 (μmol TE/g), respectively. Our work provides a detailed examination of region and cultivar effects on potential of ARs in wheat bran and the results can be used for screening and breeding purposes.

1. Introduction

Wheat, maize and rice represent approximately 2 billion tons of world total annual yields. From those three crops, wheat is the most widely grown and consumed cereal by humans (Shewry et al., 2010). Phytochemicals present in grains have potential to reduce diseases such as cardiovascular diseases (Anderson, 2004), diabetes (Montonen et al., 2003) and cancer (Landberg et al., 2009b). Some of the mentioned health benefits may be attributed to antioxidant activity of phenolic compounds such as ferulic acid, other polyphenols (lignans, anthocyanins and alkylresorcinols), caretonoids and vitamin E (Fardet, 2010).

ARs are phenolic lipids, called resorcinol lipids, present in the cereal bran of mainly rye, triticale and wheat (Athukorala et al., 2010; Ross and Kochhar, 2009). ARs are located in the intermediate layers of pericarp and testa in the grain (Landberg et al., 2008). The structure of ARs is 1, 3-dihydroxy-5-alkylbenzene derivatives with odd-numbered, mostly saturated hydrocarbon side chains in the range of 15–25 carbon atoms. ARs can be used as biomarkers, indicating the degree of endosperm extraction in wheat flour. Although lower extraction rate produces highly refined wheat flour, ARs can still exist in it because of the inevitable contamination during the separation process (Landberg et al., 2008). Also, ARs have been suggested as markers for whole grain, rye products and a biomarker for human intake of whole grain wheat and rye even though the total ARs in wheat or rye differ in cereal species, the relative homologue composition of ARs remains almost constant within the species (Chen et al., 2004).

ARs affect physicochemical properties of biological membranes due to their amphiphilic character besides their antibacterial and antifungal activity (Fardet, 2010). They have potential to modulate activity of some enzymes, and improve membrane phospholipid bilayer properties (Stasiuk and Kozubek, 2010). For instance, long chain ARs mixtures have been reported to prevent the peroxidation of fatty acids and phospholipids in liposomal membranes as well as autoxidative processes in triglycerides and fatty acids (Korycinska et al., 2009).

Wheat has significant levels of antioxidants, and among those, phenolic compounds may elicit the highest health benefits. They

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exist in free, bound and soluble conjugated forms. The most dominant free phenolic compounds are ferulic, *p*-coumaric and vanillic acids. They are found with other phenolics including caffeic, chorogenic, gentisic, syringic, and *p*-hydroxybenzoic acids (Onyeneho and Hettiarachchy, 1992). Previous studies have shown that bioactive compounds (tocopherols, sterols, alkylresorcinols, folates, phenolic acids, and fiber components) can be affected by different environmental factors (Andersson et al., 2010; Mpofu et al., 2006; Shewry et al., 2010). The interactions between wheat genotype and the environment in which wheat is grown, and possibly genotype–environment interactions can likely strongly influence the levels of grain antioxidants (Mpofu et al., 2006). Although the literature is relatively deficient in this field, Shewry et al. (2010) showed significant correlations between the contents of bioactive components (alkylresorcinols, sterols, tocols, folates, phenolic acids and fiber compounds) and environmental factors (precipitation and temperature), with even highly heritable components differing in amount between grain samples grown in different years on different sites.

ARs in cereal grain and products have been quantified using gas chromatography (GC) (Landberg et al., 2009a) and high performance liquid chromatography (HPLC)-Coularray-Based Electrochemical Detection (Ross and Kochhar, 2009). Also, ARs metabolites in human urine have been determined by gas chromatography/mass spectrometry (GC/MS) (Marklund et al., 2010).

To the best of our knowledge, there have been no reports on the relative contributions of cultivar and region effects of wheat grown in Ontario, Canada to its bioactive composition, especially ARs and its antioxidant activity. This study was carried out to determine the effects of cultivar and region on AR content and composition of 24 wheat bran samples from 6 wheat cultivars grown in 4 different regions in ON using GC/MS. Also, it was aimed to evaluate both effects on the total phenolic content (TPC) and antioxidant activity of wheat bran extracts by measuring oxygen radical absorbance capacity (ORAC) and 2,2-Dipheny-1-picryhydrazyl (DPPH) radical scavenging activity and to screen wheat rich in ARs and bioactive components for future breeding study.

2. Materials and methods

2.1. Samples

The wheat bran samples were provided by Plant Agriculture Department, Guelph University, Ridgetown, Ontario (ON), Canada. The samples were from six winter wheat cultivars including three hard red wheat; AC Morley, Harvard and Warthog and three soft red wheat; Emmit, Superior and FT Wonder. The samples were grown in 4 different regions; Bath, Nairn, Palmerstone and Rigdetown in ON during the crop year 2008. The average precipitation, maximum and minimum temperatures from May 1st to June 31st in 2008 in the above regions were 265 mm, 21.5 °C and 9.5 °C for Bath; 340 mm, 21.0 °C and 10.1 °C for Nairn; 394 mm, 19.1 °C and 8.5 °C for Palmerstone; 340 mm, 20.9 °C and 9.9 °C for Ridgetown, respectively.

Fine wheat (hard and soft) bran was prepared by abrading outer layers of grain in a Satake TM 05 laboratory scale pearler (Satake Co, Japan) to remove 10% (by weight) of bran. Prior to extraction, the bran was ground to the recommended 2 mm size (Gliwa et al., 2011) using a Thomas Wiley Mill (model ED-5, Arthur H. Thomas Co., Philadelphia, Pennsylvania, USA). Samples (24 wheat bran) were stored in sealed plastic bags and kept in the freezer (−30 °C) prior to analysis.

2.2. Materials

N–O-bis-(trimethylsilyl)-tri-fluoroacetamide with 1% trimethylchlorosilane was purchased from VWR International (Ottawa, Canada) for derivatization of samples before GC analysis. Solvents, acetone and ethyl acetate were HPLC grades and purchased from Caledon Laboratories LTC (Georgetown, ON, Canada). The AR standards (C15:0, C17:0, C19:0. C21:0, C23:0 and C25:0) in addition to C22:0 (Internal standard) were purchased from ReseaChem GmbH (Burgdorf, Switzerland). Mono-and dibasic potassium phosphate, Fluorescein, Trolox, Rutin, 2,2′-Azobis (2-methylpropionamidine) dihydrochloride (AAPH), Folin–Ciocalteau reagent, α-tocopherol and 2,2-Dipheny-1-picryhydrazyl radical (DPPH) were obtained from Sigma (Oakville, ON, Canada). Ferulic acid standard was purchased from Fluka Analytical (Sigma) (Oakville, ON, Canada). Sodium carbonate was obtained from Church and Dwight Canada Corp. (Mississauga, ON, Canada).

2.3. Extraction of alkylresorcinols (ARs)

ARs in wheat bran samples (1 g) were extracted with acetone in a 1:40 (w/v) ratio for 24 h, by continuous stirring (Stirrer-VWR, Corning®, VMS-C4) at room temperature (Gliwa et al., 2011) and filtered using Whatman double filter paper (number 1). The precipitate was discarded and the acetone was completely evaporated to dryness from the supernatant using a Rotavapor (Buchi–Brinkman, R110, Switzerland). The extract was stored in the dark at −20 °C until further analysis of TPC, DPPH, ORAC and GC/MS. All measurements were conducted in triplicate.

2.4. GC/MS analysis

GC/MS analysis was performed according to the method of Athukorala et al. (2010). Extracts (200 μL) were spiked with 200 μL of internal standard AR C22:0 (0.5 mg/ml). The solvent was evaporated under nitrogen, and were derivatized with trimethylsilyl (TMS) using 200 μL of bis-(trimethylsilyl)-trifluoroacetamide containing 1% trimetylchlorosilane (TMSC) and 100 μL of ethyl acetate. The reaction mixture was heated at 75 °C for 30 min and excess of reagent was then evaporated under nitrogen and re-dissolved in ethyl acetate for GC/MS analysis. The GC/MS analysis was conducted with a Hewlett Packard HP 6890 Plus (GC) and Model G1540A equipped with a network mass selective detector. A DB-17HT high-temperature capillary column was used (30 m × 0.25 mm I.D., 0.1 μm film thicknesses, J & W Scientific, Folsom, California). Helium was used as the carrier gas with a flow rate of 1.3-mL/min and split injection by a 10:1 ratio. The initial oven temperature of 150 °C was held for 2 min and the temperature was increased by 10 °C/min to 320 °C. Upon reaching 320 °C, it was held for 7 min and the temperature was increased to 325 °C for 1 min. A Hewlett Packard HP 5973 (MS) was operated in the electron ionization (EI) mode at 70 eV, a source temperature of 280 °C and temperature of 150 °C, in the scan range of *m*/*z* 35 to 350. Data were collected by ChemStation software (Shawnee, KS, USA). Identification of components was done by comparing their spectral data with ARs-standards (C15:0 to C25:0). The MS was used to confirm the presence of AR homologues by their molecular ion peaks at *m*/*z* 320 (C15:0), 348 (C17:0), 376 (C19:0), 404 (C21:0), 432 (C23:0) and 460 (C25:0). Data were compared against the NIST (v.02) and Wiley (v.138) libraries (Palisade Corp., Newfield, New York) (Athukorala et al., 2010).

2.5. Measuring total phenolic content (TPC) of wheat bran extracts

TPC was determined by using modified procedures of the Folin–Ciocalteu method of Gao et al. (2002). Folin–Ciocalteu reagent was diluted 10 times with deionized water. The extract, 200 μL was added to 1.9 mL of freshly diluted Folin–Ciocalteu reagent. Then, 1.9 mL of sodium carbonate solution (60 g/L) was added to the mixture. It was left for 2 h incubation at ambient temperature and the absorbance of