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Genotype, environment, and their interactions on the phytochemical compositions and radical scavenging properties of soft winter wheat bran

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ABSTRACT

The effects of genotype (G), growing environment (E), and their interactions (G × E) on the phytochemical compositions and radical scavenging properties of wheat bran were investigated using ten soft winter wheat (*Triticum aestivum* L.) varieties grown in four locations. In general, E had stronger influence on the selected health beneficial components and antioxidant properties of soft winter wheat brans than G or G × E ($P < 0.001$). E had a strong impact on α-tocopherol, δ-tocopherol, total tocopherols, total phenolic content (TPC), total soluble ferulic acid, and $ABTS^{\bullet+}$ cation and $DPPH^{\bullet}$ radical scavenging capacities ($P < 0.001$). The results also showed that each soft wheat bran component or radical scavenging property may respond to individual environmental factors differently. For the first time, the present study showed that E, G, and G × E differed in their effects on the selected health components and antioxidant properties of soft red winter wheat bran.

1. Introduction

Wheat (*Triticum aestivum* L.) is generally categorized into hard and soft classes according to its agronomic and end-use attributes, and its consumption is increasing world-wide (USDA, 2013a). Soft wheat, especially soft red wheat, accounts for 15–20 percent of total production in the United States (USDA, 2013b). Growing evidence indicates that wheat and wheat-based foods may contain health-beneficial components including natural antioxidants such as ferulic acid (Hejtmánková, Lachman, Hejtmánková, Pivec, & Janovská, 2010; Liyana-Pathirana & Shahidi, 2006; Mateo Anson, Van den Berg, Havenaar, Bast, & Haenen, 2008), lutein (Adom, Sorrells, & Liu, 2005) and α-tocopherol (Lachman, Hejtmánková, & Kotíková, 2013; Zhou, Su, & Yu, 2004), and potential cholesterol-lowering components (Cheng, Zhou, Luther, Yin, & Yu, 2008).

It was noted that the health beneficial components and properties of wheat grain might depend on both genetic and environmental conditions (William, O'Brien, Eagles, Solah, & Jayasena, 2008). For instance, total phenolic content (TPC), $DPPH^{\bullet}$ scavenging capacity, and the contents of vanillic, syringic and ferulic acids in hard spring wheat were more altered by growing environment (E), while genotype (G) contributed more to caffeic and *p*-coumaric acid contents (Mpofu, Sapirstein, & Beta, 2006). Also noted was that the genotype and growing environment interaction (G × E) contributed up to 6.71% influence to accumulation of the tested health components under the experimental conditions. Also in 2006, our previous study showed that TPC, levels of individual phenolic acids and scavenging capacities against $ABTS^{\bullet+}$ and $O_2^{\bullet-}$ of hard wheat bran were primarily controlled by environment, with E being generally a much greater source of variation than G and G × E (Moore, Liu, Zhou, & Yu, 2006). Recently, our laboratory investigated the effect of G, E and G × E on the antioxidant properties and chemical compositions of soft winter wheat flour. The results showed that E had the largest effect on scavenging activity against oxygen, hydroxyl and $ABTS^{\bullet+}$ radicals as well as total carotenoid contents, while G × E interaction had a larger effect on the level of total tocopherols (71.6%) (Lv et al., 2013).

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It is well accepted that natural antioxidants are condensed mostly in the bran fraction of wheat grains (Moore et al., 2005). As a continuation of our recent study on soft red winter wheat flour (Lv et al., 2012, 2013), the present study investigate for the first time whether and how G, E, and G × E may alter health beneficial components and properties of soft red winter wheat bran, including their phenolic acid compositions, carotenoid and tocopherol profiles, and scavenging capacities again hydroxyl ($HO^{\bullet}$), peroxyl (ORAC), $ABTS^{\bullet+}$ cation and $DPPH^{\bullet}$ radicals. The environmental factors included precipitation and temperature stress. The understanding of the G, E and G × E effects on wheat health properties could also be used for improving the breeding efforts and agricultural approaches to produce soft wheat grain and bran rich in selected health components and properties to meet market needs.

2. Materials and methods

2.1. Materials

Ten soft red winter wheat (*T. aestivum* L.) cultivars, SS520, SSMPV57, SS5205, USG3555, USG3665, USG3315, Branson, Shirley, Jamestown, and Chesapeake were included in the study. These genotypes, that represent a sample of elite commercial cultivars currently grown in the mid-Atlantic, were grown in the field during the 2011 growing season at four testing locations (Clarksville, Keedysville, Poplar Hill (Quantico) and Wye (Queenstown)) in Maryland, in yield trial plots 4 m long by 1 m wide at a density of approximately 350,000 plants/ha. Plots were planted following a crop of corn in October 2010. Plots were fertilized with an autumn application of 16 kg/ha of nitrogen, 40 kg/ha of phosphorus, and 80 kg/ha of potassium. Additionally, 30–80 kg/ha of nitrogen was applied in March or April 2011 (depending on location). Grain from the field plots was mechanically harvested, threshed, and cleaned of debris prior to laboratory testing.

2.2. Chemicals and reagents

Disodium ethylenediaminetetraacetate (EDTA), 2, 2'-bipyridyl, 2, 2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$), fluorescein (FL), lauryl sulfate sodium salt, sodium hydroxide, ethyl ether, ethyl acetate, 6-hydroxy-2, 5, 7, 8- tetramethylchroman-2-carboxylic acid (Trolox), tocopherols (α-, δ-, and γ-), ascorbic acid, and β-carotene were purchased from Sigma–Aldrich (St. Louis, MO, USA). Iron (III) chloride, ABTS™ chromophore, diammonium salt and thirty percent ACS-grade hydrogen peroxide were obtained from Fisher Scientific (Fair Lawn, NJ, USA). 2, 2'-azinobis (2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals (Richmond, VA, USA). Ultrapure water was used for all experiments, which was prepared using an ELGA Purelab ultra Genetic polishing system with <5 ppb TOC and resistivity of 18.2 mΩ (Lowell, MA, USA). All other chemicals and solvents were of the highest commercial grade and used without further purification.

2.3. Preparation and extraction of soft wheat bran

Each wheat bran sample was ground to a particle size of 0.42 mm using a handheld coffee grinder. Half gram of ground wheat bran sample was extracted with 5 mL of 895.5 g/L acetone for 24 h under nitrogen at ambient temperature. The acetone extracts were used for estimating total phenolic content (TPC) and radical scavenging capacities (HOSC, RDSC, ORAC and $ABTS^{\bullet+}$ scavenging capacity).

2.4. Total phenolic content (TPC)

The TPC of wheat bran was determined using the Folin–Ciocalteu reagent according to a laboratory procedure described previously (Yu, Haley, Perret, & Harris, 2002), with gallic acid as the standard.

2.5. Total soluble ferulic acid content of soft wheat bran

Each wheat bran sample was analyzed for its total soluble ferulic acid including soluble free and conjugated ferulic acids according to the laboratory method described by Moore et al. (2005). In brief, ground wheat bran was extracted with acetone/methanol/water (7:7:6, v/v/v) and the supernatant was collected. Free ferulic acid was extracted with ethyl ether and ethyl acetate (1:1, v/v) at pH 2. After evaporating the organic phase under nitrogen, each extract was re-dissolved in methanol and filtered through 0.20 μm membrane filter, and subjected to HPLC analysis. The mobile phase A consisted of acetic acid/H_2O (2:98, v/v) and mobile phase B consisted of acetic acid/acetonitrile/H_2O (2:30:68, v/v/v). Elution was programmed from 10 to 100% B in 42 min with a flow rate of 1.0 mL/min. Quantification was based on the area under the peak of external standards. The results were expressed as μg/g of wheat bran on a dry weight basis.

2.6. Carotenoid composition

Two hundred mg of ground wheat bran was extracted with 10 mL of methanol/tetrahydrofuran (1:1, v/v) for 15 h at ambient temperature. After removing solvent, the residues were re-dissolved in 2 mL of methanol/acetonitrile/iso-propanol (54:44:2, v/v/v) for carotenoid analysis using HPLC. HPLC separation was accomplished using a Shimadzu LC-20AD with an autosampler, a Phenomenex C18 column (4.6 mm i.d. ×250 mm, 5 μm particle size) at 25 °C and a UV-VIS detector at 450 nm (Moore et al., 2005). Water was the solvent A and methanol/acetonitrile/iso-propanol (54:44:2, v/v/v) as solvent B. The elution was achieved by: 1) linear gradient from 95% to 99% of solvent B in the first 10 min, 2) 99% of solvent B for 10 min, and 3) linear gradient from 99% to 95% of solvent B for the last 5 min. The flow rate was 1.0 mL/min and 20 μL of each standard or sample was injected. Peak area was used for quantification. The results were expressed as μg/g of wheat bran on a dry weight basis.

2.7. Tocopherol content

HPLC analysis was accomplished using a Shimadzu LC-20AD with an autosampler and an UV-VIS detector, and a Phenomenex C18 column (4.6 mm i.d. ×250 mm, 5 μm particle size) at 25 °C according to a previously described protocol (Zhou, Yin, & Yu, 2005). The tocopherols were separated using an isocratic elution with a flow rate of 1.5 mL/min and a mobile phase 1% solvent A (water) and 99% solvent B (acetonitrile). The results were reported as μg/g of bran on a dry weight basis.

2.8. Determination of radical scavenging capacities

Hydroxyl radical ($HO^{\bullet}$) scavenging capacity (HOSC) was examined according to a previously reported laboratory procedure using a Victor^3 multi-label plate reader (PerkinElmer, Turku, Finland) (Moore, Yin, & Yu, 2006). The relative $DPPH^{\bullet}$ radical scavenging capacity (RDSC) was determined according to a previously described laboratory procedure (Cheng, Moore, & Yu, 2006). In addition, oxygen radical absorbance capacity (ORAC) was measured following a previously reported laboratory protocol using a Victor^3