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Wheat bran particle size influence on phytochemical extractability and antioxidant properties

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ABSTRACT

It is unknown if particle size plays a role in extracting health promoting compounds in wheat bran because the extraction of antioxidant and phenolic compounds with particle size reduction has not been well documented. In this study, unmilled whole bran (coarse treatment) was compared to whole bran milled to medium and fine treatments from the same wheat bran. Antioxidant properties (capacity, ability, power), carotenoids and phenolic compounds (phenolic acids, flavonoids, anthocyanins) were measured and compared. The ability of whole bran fractions of differing particle size distributions to inhibit free radicals was assessed using four *in vitro* models, namely, diphenylpicrylhydrazyl radical-scavenging activity, ferric reducing/antioxidant power (FRAP) assay, oxygen radical absorbance capacity (ORAC), and total antioxidant capacity. Significant differences in phytochemical concentrations and antioxidant properties were observed between whole bran fractions of reduced particle size distribution for some assays. The coarse treatment exhibited significantly higher antioxidant properties compared to the fine treatment; except for the ORAC value, in which coarse was significantly lower. For soluble and bound extractions, the coarse treatment was comparatively higher in total antioxidant capacity (426.72 mg ascorbic acid eq./g) and FRAP value (53.04 μmol $FeSO_4$/g) than bran milled to the finer treatment (314.55 ascorbic acid eq./g and 40.84 μmol $FeSO_4$/g, respectively). Likewise, the fine treatment was higher in phenolic acid (7.36 mg FAE/g), flavonoid (206.74 μg catechin/g), anthocyanin (63.0 μg/g), and carotenoid contents (beta carotene, 14.25 μg/100 g; zeaxanthin, 35.21 μg/100 g; lutein 174.59 μg/100 g) as compared to the coarse treatment. An increase of surface area to mass increased the ORAC value by over 80%. With reduction in particle size, there was a significant increase in extracted anthocyanins, carotenoids and ORAC value. Particle size does effect the extraction of phytochemicals.

1. Introduction

Wheat flour milling separates the endosperm from bran to produce flour. Wheat bran is a by-product of conventional milling that contains hemicellulose, protein, cellulose, and micronutrients at relatively high concentrations; namely as 41–60% nonstarch polysaccharides (26% are arabinoxylans), 15–20% protein and 10–20% residual starch (Amrein, Gränicher, Arrigoni, & Amadò, 2003).

Phytochemicals are bioactive plant compounds produced in edible plants. Wheat bran has many health benefits and health promoting phytochemicals such as phenolic acids, flavonoids and carotenoids (Muir et al., 2004). Several subclasses exist within the numerous chemical compounds that represent the wheat phytochemicals (alkylresorcinols, phenolic acids, etc.). For example, anthocyanins are a type of flavonoid, while flavonoids are a type of water soluble phenol found in plants. Phytochemicals are also important sources of exogenous antioxidants in the diet (Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002).

Wheat bran contains several phytochemicals that could be absorbed during digestion, yet are unavailable due to delivery structure and transit time in the human gastrointestinal tract (Brownlee, 2011). After mastication, wheat bran particle size is scarcely altered, nor greatly digested prior to the large intestine. Mostly intact wheat bran travels to the distal colon where it is fermented (Brownlee, 2011), therefore, the initial particle size is important.

It is thought that processing may release bound phytochemicals from grains (Fulcher & Duke, 2002), but the concentration and extractability of phytochemicals in relation to the exposed surface area is not well documented. Previous studies on bran particle size have examined dough and baking properties of bran ground to coarse medium and fine samples from the same stock material (Zhang & Moore, 1999). Multiple studies have investigated the

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bran particle size effects on digestion, noting that a reduced particle size usually coincides with a decrease in total stool water (Brownlee, 2011). However, limited data are available on the bioavailability of biochemical components and effects of particle size distribution even though the interest is high in this subject due to the high content of fibre and bioactive compounds in wheat bran. Rosa, Barron, Gaiani, Dufour, and Micard (2013) reported that ultra-fine grinding increases the antioxidant capacity of wheat bran without any prior extraction. Hemery et al., (2010) showed that the reduction of particle size was correlated with an increase in the bioaccessibility of phenolic acids. Micronization of aleurone increased its antioxidant activity (Zhou, Laux, & Yu, 2004). Investigations of the wheat bran antioxidant properties with reduction by ball milling has been reported using a reduced particle size dietary fibre source derived from wheat bran (Zhu, Huang, Peng, Qian, & Zhou, 2010), while the variation in tocopherols and tocotrienols with reduction in wheat bran particle size has also been observed (Engelsen & Hansen, 2009). Such research leads to questioning whether phytochemicals, such as antioxidants, are more extractable in wheat bran with a reduction in its particle size. The objective of this research was to determine if particle size distribution of whole wheat bran affects the phytochemical extractability and antioxidant properties as determined by *in vitro* testing. As no single measure of antioxidant concentration can express the ability, activity and capacity of antioxidants present, due to the chemical diversity of antioxidants (Ou et al., 2002), several commonly used 'total antioxidant' *in vitro* models were utilised to determine the ability of extracts from wheat bran to scavenge free radicals and reactive oxygen species. In this study, we used four *in vitro* methods, namely, diphenylpicrylhydrazyl radical-scavenging activity, ferric reducing/antioxidant power (FRAP) assay, oxygen radical absorbance capacity (ORAC), and total antioxidant capacity to determine the ability of whole bran fractions of differing particle size distributions to inhibit free radicals. In addition, free and bound phenolics, flavonoid, anthocyanin, and carotenoid were measured and compared in one study. As whole grain (reconstituted grain with the correct proportion of endosperm, flour and germ) and whole wheat (wheat berry ground to flour consistency without separation of the components) are commonly found in packaged foods, it is important to determine if a reduction in particle size may increase the proportion of available phytochemicals in wheat bran.

2. Materials and methods

2.1. Wheat bran samples

Mixed Kansas (*Triticum aestivum* L.) hard red winter wheat (Likes, Madl, Zeisel, & Craig, 2007) from the 2010 crop year was conditioned to 16% moisture and milled using the Hal Ross Mill (Kansas State University, Manhattan, KS) at a 72% extraction rate (0.52% ash). The milling system used has been previously described (Likes et al., 2007). All wheat bran was collected from one outlet after the purifier, during a single mill run. Unmilled, whole wheat bran acted as the control and is referred to throughout as 'the coarse treatment'. The coarse treatment was collected, kept in tinted, air-tight containers and stored at 4 °C for no more than six months prior to analysis. A portion of the coarse treatment was used for particle size profiling to provide a reference for processing two additional treatments and as a sample to determine multiple analyses. The remainder of the coarse treatment was divided in half to make up two ground treatments. For preparation of the two treatments, a corrugated (20/22 per square inch; 0.013/0.014 per square metre; 2.5:1 differential) Ross experimental roller mill (serial # 915, size 9×6; Oklahoma City, OK) was employed (Experimental Milling Lab, Manhattan, KS) with an experimental gap between the rolls, milling is described as follows. The first treatment, defined and referred to throughout as 'the fine treatment', was milled to the finest whole wheat bran particle size distribution achievable. The gap is defined as when the rolls were adjusted to just above zero gap, where the corrugated rollers were touching (as noted by sound), but not stopping the rotation of the rolls. Once the rolls were adjusted, the treatment was milled via three passes. Three passes were noted to be efficient with the set gap; and three passes were incorporated to reduce the wheat bran to the desired size, without damaging the product or equipment, and without applying too much energy/heat to the bran. Based on the particle profile, the second treatment was milled so the bran particle size was in-between the particle size distribution of the coarse and fine treatments. This treatment was designated as 'the medium treatment'. The medium treatment was prepared by increasing the gap between the rolls slightly so that the medium treatment visually differed from the coarse and fine treatments. The medium treatment was also milled via three passes through the same Ross experimental mill. All treatments were defined by sieving (Table 1). The particle size reduction schematic and resulting bran samples are described in Fig. 1.

2.2. Chemicals

All chemicals, reagents, and standards were ACS or HPLC grade. Ascorbic and phenolic acid standards were purchased from Sigma–Aldrich, Inc. (St. Louis, MO). Carotenoid standards were obtained from DSM (DSM Nutritional Products, Boulder, CO).

2.3. Particle size determination

All the whole wheat bran samples were sieved on a standard Tyler Rotap sieve shaker (W. S. Tyler, Mentor, Ohio). To determine whole wheat bran size distributions from milling, coarse treatments were sieved through 900, 750, 500, and 355 μm mesh screens. To determine the whole wheat bran size distributions from grinding, medium treatments were sieved through 1041, 500, 355, and 240 μm mesh screens. The fine treatments were sieved through 355, 200, 150, and 100 μm mesh screens. The pan is noted as any material that passed through the sieve mesh dimensions.

2.4. Soluble and bound phenolic compounds

A soluble and bound phenolic compound extraction was performed as previously described (Adom, Sorrells, & Liu, 2005). Sample (1.000 g) were extracted (10 min, under constant stirring) with 10 ml 80% methanol (v/v) at 25 °C. Subsequently, the extract was removed, pooled with extract from repeating the procedure two addition times on the residual pellet. Pooled extracts were evaporated under continuous nitrogen gas flush. Each extracted sample was lyophilized, weights recorded, and dissolved in 5 ml methanol prior to analysis. To obtain the bound phenolics, the above pellet was hydrolysed with excess 2 M sodium hydroxide at 25 °C for 1 h under nitrogen gas flush, neutralized with an equal amount of 2 M hydrochloric acid, and extracted with pure hexane. All hexane was removed and the hydrolysate was extracted with ethyl acetate (five times). Ethyl acetate extracts were pooled, evaporated to dryness under continuous nitrogen gas flush, dissolved in 10 ml methanol, and stored at −20 °C until use. Determination of total phenolic content (TPC) in each fraction utilised the reduction of Folin–Ciocalteu reagent in the presence of phenolates, measured spectrophotometrically on a Perkin–Elmer Lambda 800 UV–Vis spectrophotometer (Perkin–Elmer, Inc., Waltham, MA). A 125 μl ferulic acid standard solution or extract sample was added to