Wheat bran particle size influence on phytochemical extractability and antioxidant properties

Lauren Renee Brewer, Jittawan Kubola, Sirithon Siriamornpun, Thomas J. Herald, Yong-Cheng Shi

A R T I C L E   I N F O

Article history:
Received 7 May 2013
Received in revised form 16 October 2013
Accepted 23 November 2013
Available online 8 December 2013

Keywords:
Antioxidant
Particle size
Phenolic compounds
Wheat bran

A B S T R A C T

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 FeSO4/g) than bran milled to the finer treatment (314.55 ascorbic acid eq./g and 40.84 µmol FeSO4/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.

© 2013 Elsevier Ltd. All rights reserved.

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
Wheat 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 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
125 μl Folin–Ciocalteu reagent, brought up to volume with 0.5 ml deionized water in a test tube and vortex-mixed. Samples were held for 6 min before adding 1.25 ml 7% sodium carbonate and adjusting the final volume to 3 ml with deionized water. After a 90 min incubation period (25°C), absorbance was measured at 760 nm against the blank and compared with known ferulic acid standards for quantification; expressed as ferulic acid equivalents (FAE) per gram of bran. Ferulic acid is a common standard in TPC analysis, (Adom et al., 2005) and similar trends would be seen with alternate phenolic compound standards.

2.5. Flavonoid and carotenoid extraction

Flavonoids and carotenoids were extracted as previously described (Adom et al., 2005). Each sample (600 mg) was weighed and blended with 60 mg magnesium carbonate prior to rapid extraction with 2 ml 1:1 (v/v) methanol/tetrahydrofuran mixture in a water bath at 75°C for 5 min in a loosely closed screw-capped test tube. Extracts were cooled and centrifuged at 2500 g for 5 min, and the organic phase was removed from the pellet. The extraction procedure was repeated two additional times on the pellet. Pooled organic phases were dried with 1 g anhydrous sodium sulphate and evaporated under continuous nitrogen gas flush (35°C). Residues were dissolved in 1 ml methanol/tetrahydrofuran for storage (−20°C) and analysis, and were analysed within two weeks.

2.6. Total flavonoid content

The determination of total flavonoid content was conducted as previously described (Liu et al., 2002). Extracts from the flavonoid extraction (0.25 ml) were mixed with 1.25 ml distilled and 75 μl of 5% sodium nitrite solution, subsequently the tubes were held at 25°C for 6 min. After, 150 μl 10% aluminium chloride was added in each test tube and held at 25°C for 5 min. Lastly, 0.5 ml 1 M sodium hydroxide and 2.5 ml with distilled water were added and vigorously mixed. Samples were immediately measured (510 nm) against a blank on a spectrophotometer. Total flavonoid content was calculated as microgram of catechin equivalent (CE) per gram of bran against a standard curve of catechin (Liu et al., 2002).

2.7. Total anthocyanin content

The extraction and determination of anthocyanin content was conducted as previously described (Abdel-Aal, Young, & Rabalski, 2006). Samples (3.000 g each) were extracted twice by vigorous mixing with 24 ml acidified methanol [1 N hydrochloric acid (85:15, v/v)] for 30 min. The apparent pH was adjusted to 1.0 prior to timing and rechecked at 15 and 30 min of extraction. Extracts were centrifuged at 21,000 g (4°C) for 20 min and refrigerated for 2 d to allow the compounds to precipitate. After, extracts were centrifuged 21,000 g (4°C) for 20 min and concentrated to 2 ml under nitrogen gas flush. The concentrated supernatant was added to a 50-ml volumetric flask and made up to volume with acidified methanol. Absorbance was measured on a spectrophotometer (535 nm), and anthocyanin content calculated as microgram equivalents of cyanidin 3-glucoside per gram of bran (Abdel-Aal et al., 2006).

2.8. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay

The determination of DPPH radical absorbance was conducted as previously reported (Yu et al., 2002). DPPH reagent, consisting of DPPH (0.004%) in methanol [1 N hydrochloric acid (85:15, v/v)] for 30 min. The apparent pH was adjusted to 1.0 prior to timing and rechecked at 15 and 30 min of extraction. Extracts were centrifuged at 21,000 g (4°C) for 20 min and refrigerated for 2 d to allow the compounds to precipitate. After, extracts were centrifuged 21,000 g (4°C) for 20 min and concentrated to 2 ml under nitrogen gas flush. The concentrated supernatant was added to a 50-ml volumetric flask and made up to volume with acidified methanol. Absorbance was measured on a spectrophotometer (535 nm), and anthocyanin content calculated as microgram equivalents of cyanidin 3-glucoside per gram of bran (Abdel-Aal et al., 2006).

### Table 1

<table>
<thead>
<tr>
<th>Sieve μm</th>
<th>Coarse whole bran %</th>
<th>Sieve μm</th>
<th>Medium whole bran %</th>
<th>Sieve μm</th>
<th>Fine whole bran %</th>
</tr>
</thead>
<tbody>
<tr>
<td>900</td>
<td>91</td>
<td>1041</td>
<td>2</td>
<td>355</td>
<td>2</td>
</tr>
<tr>
<td>750</td>
<td>7</td>
<td>500</td>
<td>43</td>
<td>200</td>
<td>48</td>
</tr>
<tr>
<td>500</td>
<td>2</td>
<td>355</td>
<td>24</td>
<td>150</td>
<td>17</td>
</tr>
<tr>
<td>355</td>
<td>0</td>
<td>240</td>
<td>24</td>
<td>100</td>
<td>12</td>
</tr>
<tr>
<td>Pan a</td>
<td>1</td>
<td>PAN a</td>
<td>10</td>
<td>PAN a</td>
<td>24</td>
</tr>
</tbody>
</table>

a The pan is noted as any material that passed through all sieve mesh dimensions utilised.

![Fig. 1. (a) Photographs of whole bran treatments milled from the same wheat kernels; scale bar represents 0.5 cm. The coarse treatment is described as control and stock material, with particle size varying 90–5000 μm. The medium treatment was milled from coarse bran to less than 3% over the 500 μm sieve. The fine treatment was milled from stock wheat bran material to less than 3% over the 200 μm sieve. (b) Particle size reduction schematic. Where, Analyses – the multiple analytical techniques used to quantify attributes for each particle size distribution; Fraction – the desired particle size distribution (200 or 500 μm); Pass – sending the wheat bran through the roller with the experimental gap set; and, Sieve – sieving the material with the predetermined sieves for the desired particle size distribution (as noted in Table 1).](image-url)
30 min, absorbance was tested (517 nm) on a spectrophotometer against a standard of ascorbic acid. The IC₅₀ value was used to calculate DPPH value and was defined as the concentration of the sample necessary to have 50% inhibition as determined by interpolated linear regression. DPPH values are reported as % inhibition.

2.9. Ferric reducing antioxidant power (FRAP) assay

The determination of FRAP was conducted as previously reported (Yu, Perret, Harris, Wilson, & Haley, 2003). FRAP reagent was prepared the day of analysis and kept (up to 3 h) in a water bath at 37 °C when not in use. Preparation of the FRAP reagent was as follows: acetate buffer 300 mM (pH 3.6), was added to 2,4,6-tripyridyl-s-triazine (10 mM in 40 mM hydrochloric acid) and iron(III) chloride hexahydrate. (20 mM) in a ratio of 10:1:1 (Benzie & Strain, 1999). Combined in one test tube and incubated at 37 °C for 4 min were: 1.8 ml FRAP reagent, 300 μl extract from the soluble/bound phenolic extraction, and 180 μl distilled water. The absorbance was measured (593 nm) on a spectrophotometer and reported in micromole ferrous sulphate (FeSO₄) per gram defatted bran.

2.10. Oxygen radical absorbance capacity (ORAC)

The determination of ORAC value was conducted as previously reported (Ou et al., 2002), with modification to the extraction time (1 h) and stirring equipment (stir bar and stir plate). Each sample (500 mg) was added to 20 ml hexane:dichloromethane (1:1) to extract lipophilic antioxidant constituents. Mixtures were stirred with magnetic stirring bars to turbulence at 25 °C for 1 h, under nitrogen gas flush. Extracts were removed, evaporated to dryness at 25 °C under nitrogen gas flush, and stored at −20 °C until analysis. The above lipophilic extracts were solubilized with 1 ml methanol prior to analysis. To extract hydrophilic antioxidant constituents, bran previously extracted for lipophilic compounds was mixed with acetone:water (70:30) for 1 h at 25 °C, under nitrogen gas flush. The extract was centrifuged at 12,100 g for 15 min, and stored at −20 °C until analysis. Both extracts (hydrophilic and lipophilic) were analysed utilising a Synergy 2 microplate reader equipped with Gen5TM data analysis software (Biotek Instruments Inc., Winooski, VT, USA), and reported as micromol Trolox equivalents (TE) per gram of bran (Ou et al., 2002).

2.11. Total antioxidant capacity

The determination of total antioxidant capacity was conducted as previously reported (Adom et al., 2005). In one test tube the following were incubated at 95 °C for 90 min: 0.3 ml from the soluble/bound phenolic extraction and 3 ml of reagent (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The mixtures were cooled to 25 °C, absorbance was read at 695 nm against a blank on spectrophotometer, and total antioxidant capacity was calculated against a reference of ascorbic acid and expressed as micromol equivalents of ascorbic acid.

2.12. Carotenoid analysis

The determination of carotenoid content was conducted as previously described (Adom, Sorrells, & Liu, 2003), with modifications to the interdiameter of the column. An Agilent 1200 HPLC system with a C-30 column (250 × 4.6 mm, 5 μm column, Waters Corp., Milford, MA) was utilised to quantify the carotenoid extracts. The mobile phase was composed of 75% solvent A: methanol/water (95:5, v/v), and 25% solvent B: pure methyl tert-butyl ether. The mobile phase had a measured pH of 6 and constant flow rate of 1.0 ml/min. From a 10 μl injection volume, the analytes were measured at 450 nm, under constant temperature (30 °C). Beta carotene, lutein and zeaxanthin were measured and reported as microgram of the respective carotenoid per 100 g bran.

2.13. Statistical analysis

All tests were performed in triplicate, unless otherwise noted. N is listed where n equals the number of assays. Triplicate measurements were taken for all assays. Means and standard deviations were calculated for all analyses. Significance of differences between groups was compared using column analysis of one-way ANOVA with Tukey’s post hoc test at a significance level of α: 0.05 (GraphPad, GraphPad Software Inc., La Jolla, CA). Two-tailed P values of less than 0.05 were considered to be a sign of statistical significance.

3. Results

3.1. Particle size distributions

All samples were sieved to determine particle size distribution by volume (Table 1). Sieving represented the milled ratio of desired bran fraction to overs. The particle size distribution by sieving the coarse, medium and fine treatments were rounded to the nearest percent, therefore, some total values are above 100%, but accurately display the fractions within the sample. Sieving determined over 90% of particles in the coarse treatment was greater than 900 μm.

3.2. TPC

The results of TPC extraction are found in Table 2. The order of soluble TPC for each treatment was as follows: fine > coarse > medium. The extraction yield of soluble phenolic compounds refers to free and conjugated phenolic acids extracted with 80% methanol, whereas that of bound phenolic compounds refers to alkaline-hydrolysed extract. The highest concentration of bound TPC was observed in the fine treatment (6.72 mg FAE/g of defatted bran), while bound TPC was lowest in the coarse treatment (4.73 mg FAE/g of defatted bran). An increase in TPC was observed with some reduction in particle size, however, coarse and medium treatment soluble and bound extracts did not significantly differ. On average, bound TPC was 10 times higher than soluble TPC in all fractions.

3.3. Flavonoid concentrations

The total flavonoid content reflects the available polyphenol population in the bran extracts (Table 2). The order of flavonoid content was determined as: fine > coarse ≈ medium, for all samples. The medium treatment did not significantly differ from the coarse treatment, and the highest flavonoid concentration was observed in the fine treatment (206.74 μg CE/g).

3.4. Anthocyanin concentrations

The total anthocyanin concentration reflects the available water soluble polyphenol population in the bran extracts (Table 2). The anthocyanin concentration in the whole bran was of the order as follows: fine > medium > coarse, for all samples.

3.5. DPPH radical- scavenging activity

All bran extracts showed DPPH scavenging activities. The DPPH scavenging activities of the treatments are reported in Table 3. The
ability to scavenge DPPH radicals by bran did not significantly differ for the soluble extracts. The ability to scavenge DPPH radicals by bran for the bound extracts was of the order of: medium ≈ coarse > fine, where the medium treatment did not significantly differ from the coarse treatment. As the standard, ascorbic acid was measured at 96% DPPH inhibition with this sample set.

3.6. FRAP assay

The antioxidant power of bran extracts was evaluated by FRAP assay in Table 3. The ability to reduce Fe²⁺ to Fe⁺⁺ was in the order of: coarse > fine > medium, for all soluble extracts. Soluble extract from the coarse treatment had a significantly higher FRAP value (23.84 μmol of FeSO₄/g defatted bran) than the other treatments, while the extract from the medium treatment had the lowest FRAP value (8.93 μmol of FeSO₄/g defatted bran). FRAP values for bound extracts were as follows: coarse > fine > medium.

3.7. ORAC assay

The ORAC assay was reported for all treatments as TE within the extract in Table 3. For hydrophilic and lipophilic extracts, the ORAC values were of the order of fine ≈ medium > coarse. The fine and medium treatment hydrophilic and lipophilic extracts were not significantly different. TE of reduced particle size whole bran samples was significantly higher than the coarse treatment.

3.8. Total antioxidant capacities

The total antioxidant capacity values are shown in Table 3. The order of the total antioxidant capacity for the soluble extraction was of the order of: coarse > medium > fine. The highest total antioxidant capacity was observed in the coarse treatment (146.20 mg/g of defatted bran). The order of total antioxidant capacity for the bound extraction was as follows: medium > coarse > fine. Bound total antioxidant capacity was highest in the medium treatment (319.17 mg/g of defatted bran).

3.9. Carotenoid concentration

The carotenoid concentrations are reported in Table 4. All bran samples had detectable levels of all three carotenoid standards used during this experiment. Beta carotene was detected in the order as follows: medium > fine > coarse, for all samples. For zeaxanthin, results were in the order as follows: fine > medium > coarse. The highest concentrated carotenoid was lutein. Whole bran samples contained lutein in the order as follows: fine > coarse > medium.

4. Discussion

The importance of bran particle size on extraction behaviour in wheat bran was confirmed. The relationship between the particle size distribution and several antioxidant analyses was demonstrated and led to the suggestion of the importance of sizing in functional food ingredient claims. The particle size distribution of the coarse treatment (approximately 91% particles more than 900 μm) and the minimum achieved grind for milled treatments

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Phenolic compound contents in whole wheat bran extracts as function of particle size distribution.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole bran composition</td>
<td>Phenolic compounds[^a]</td>
</tr>
<tr>
<td></td>
<td>Soluble</td>
</tr>
<tr>
<td>Coarse</td>
<td>0.45 ± 0.02b</td>
</tr>
<tr>
<td>Medium</td>
<td>0.37 ± 0.04b</td>
</tr>
<tr>
<td>Fine</td>
<td>0.64 ± 0.05a</td>
</tr>
</tbody>
</table>

[^a] Data with letter codes are not significantly different (p > 0.05); n = 3.
[^b] Total phenolic contents are expressed as ferulic acid equivalents (FAE).
[^c] Total flavonoid contents expressed as catechin equivalents (CE).
[^d] Total anthocyanin content expressed as equivalents of cyanidin 3-glucoside.

<table>
<thead>
<tr>
<th>Bran</th>
<th>Carotenoid concentrations (μg/100 g)[^a]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beta carotene</td>
</tr>
<tr>
<td>Coarse</td>
<td>6.11 ± 0.05c</td>
</tr>
<tr>
<td>Medium</td>
<td>17.64 ± 0.13a</td>
</tr>
<tr>
<td>Fine</td>
<td>14.25 ± 0.12b</td>
</tr>
</tbody>
</table>

[^a] Column data with like letters are not significantly different (p > 0.05); n = 3.

[^b] % Inhibition was calculated using IC₅₀ value calculate DPPH value. It is defined as the concentration of the sample necessary to cave 50% inhibition, interpolated by linear regression.
[^c] FRAP – Ferric reducing/antioxidant power.
[^d] ORAC – Oxygen scavenging antioxidant capacity, where TE is Trolox equivalent.
 (~200 μm) defined the samples chosen for this study, as no previous research of this nature has been conducted at the 100 μm scale. Names were provided for each fraction (coarse, medium, and fine) due to the unavoidable distribution from grinding heterogeneous material (Noort, van Haaster, Hemyer, Schols, & Hamer, 2010), and as no standards of identity are available for milled whole bran. Coarse and fine bran have been previously defined as unmilled bran and bran milled (equipment not specified), to 0.35–0.59 mm particles ( de Silva & Badiale-Furlong, 2009). However, in the current study, all whole bran samples were sieved utilising several appropriate mesh sizes to more accurately display the particle sizes of the various whole bran samples, as composition and size distribution were found to be important when conducting assays with heterogeneous materials, such as with wheat bran (Noort et al., 2010).

4.2. Phytochemicals with antioxidant ability

The relationship between several antioxidant analyses and phenolic quantifications was demonstrated and suggests the importance of size in extraction assays of fibrous material. Phenolic compounds are concentrated in the cell walls of the bran, however it was unexplored whether antioxidants were altered or disrupted due to grinding. After multiple analyses of three particle size distributions, only some constituents were further extracted with the reduction in particle size, as some assays are more sensitive to certain compounds (Huang, Ou, & Prior, 2005). A previous report noted some cases where the biochemical composition of sterols, folates and alkylresorcinols were not altered with changes in milling conditions; while, compounds such as phytic acid and ferulic constituents had some statistical differences (Hemyer et al., 2011), which was the case in the current study.

As some differences were seen in the current study, wheat bran fraction and composition should be considered in product formulations. Hemyer et al. (2007) determined that multiple dry milling processes can reduce the wheat bran particle size, therefore the results seen in the current study may not be observed when using alternative milling techniques. It is noted that the energy and aggregation inherently present in conventional milling may enhance some micronutrients of within wheat and inversely destroy others (Anson, Hemyer, Basta, & Haenen, 2012).

The antioxidative values are dependent on the assay used (Roy et al., 2010). Comparative results on the grain antioxidant properties are available for wheat bran fibre samples based on TPC, FRAP, and DPPH scavenging capacity (Zhu et al., 2010). Wheat bran before and after micronization, showed an increase in chelating activity, reducing power and TPC after size reduction (Zhu et al., 2010). However, DPPH decreased with increased surface area to mass and their material was lower in TPC than that of unaltered wheat bran before and after ultrafine grinding. We suspect that the redox potential of DPPH differs from that of molybdenum (VI) within the Folin–Ciocalteu reagent, which would account for the fact that DPPH and TPC do not necessarily follow the same trend. In the current study, multiple antioxidant assays were performed and DPPH inhibition also did not increase with particle size reduction. However, in contrast to Zhu et al. (2010), soluble and bound TPC were the highest for the fine treatment, and we determined that an increased accessible surface area, as particle size decreases, did affect TPC but not the DPPH values determined for wheat bran.

The coarse treatment TPC (soluble and bound) was similar to previously published results using similar extraction methods (Liyana-Pathirana & Shahidi, 2006). These data were in agreement with previous reports indicating that extracts with the highest TPC showed the greatest antioxidant properties. McCarthy et al. (2012) noted that extracts with high TPC could have the greatest protection against oxidant induced DNA damage.

The Folin–Ciocalteu reagent used in the TPC method measures TPC without distinguishing between phenolic structures (Adom et al., 2003). Thus, Folin–Ciocalteu reagent may also react with sugars and peptides, many of which are soluble in aqueous solutions and therefore were suspected to partly account for the soluble extract quantities found in this study. The value calculated from TPC is in FAE, yet the method measures reducing capacity, and hence, the antioxidant properties of the material (Huang et al., 2005). Therefore, the current study specifically measured phenolic compounds of importance to the bran layers by determining the flavonoid and anthocyanin concentrations. The determined flavonoid concentrations for the coarse treatment were similar to that of previous determinations of unmilled wheat bran (Feng & McDonald, 1989) and bran/germ have been reported (Adom et al., 2003, 2005), although the current study is the first investigation monitoring multiple whole wheat bran particle size distributions and flavonoid content. A previous study determined that the total flavonoid content of bran/germ [740–940 μmol of catechin equiv/100 g] was 10–15-fold higher than that of the flour and contributed 78% of the total flavonoid content of whole grain (Adom et al., 2005). We can assume that changes in extractability of flavonoids could carry over to products containing bran at various particle sizes. Flavonoids have been shown to exhibit potent antioxidant and anticancer activity and are a source of antioxidants in the diet (Ou et al., 2002).

In the current study, the coarse and medium treatments were not significantly different in their flavonoid content, yet their anthocyanin concentrations were significantly different. Differences in these subclasses of phenolic compounds may be due to their extractions or the chemistry that differentiates other flavonoids from anthocyanins (Wolf & Liu, 2008). The anthocyanin concentration of blue wheat bran [495.5 μg/g (Abdel-Aal et al., 2006)] is significantly higher than the in bran from hard red winter wheat analysed in the current study. On the other hand, the anthocyanin concentrations determined in this study were comparatively low (Abdel-Aal et al., 2006; Hosseinian, Lia, & Beta, 2008). Anthocyanins have been noted as antioxidants, acting by multiple mechanisms in humans (Hosseinian et al., 2008).

In this study, multiple antioxidant assays were utilised on all samples to account for the different reaction mechanisms of the antioxidants. Antioxidant quantifications are mechanistically based on either electron or hydrogen atom transfer between an oxidant and a free radical (Huang et al., 2005; Roy et al., 2010).

The DPPH assay measures single electron transfer to determine the antioxidant reducing capacity and is highly reproducible (Herald, Gadgil, & Tilley, 2012). The IC50 values obtained for the medium and coarse treatments suggest that less processing provides the best single electrontransfer in solution. The current results coincide with the theory that the antioxidant compounds on the surface of wheat bran may alter the particle size reduction and exposure of once protected chemical compounds (due to processing) (Fulcher & Duke, 2002).

FRAP analysis was adversely affected by particle size. The effect was determined by measuring the antioxidant power by the reducing power of the electron donating antioxidants present in the extract and determining the FRAP value (Huang et al., 2005). During the FRAP assay, a single electron is transferred from the antioxidant molecule to the oxidant. However, FRAP is nonspecific and compounds with lower redox potential than Fe2+ will initiate Fe2+ formation (Benzie & Strain, 1999). Both methods (FRAP and TPC) are measured by their reducing capacity, and previous researchers have noted that the FRAP activity correlated with the TPC of brewers' spent grain extracts (McCarthy et al., 2012), suggesting a correlation between the two assays. However, some assays did not significantly differ between the treatments. Non-linear changes with a reduction of particle size have been noted in previous studies (Kahlon, Berrios, Smith, & Pan, 2006).
ORAC measures the antioxidant properties by hydrogen atom transfer, assessing the antioxidant donating capacity (Huang et al., 2005). The soluble and bound TPC and the hydrophilic and lipophilic ORAC values did not have a similar trend (Ou et al., 2002). However, the ORAC values were in contrast to the DPPH, FRAP and total antioxidant capacity values. This may be due to the ORAC extractions are based on the hydrophilic and lipophilic properties and to the presence of lipid–soluble compounds. Roy et al. (2010) proposed that extracts exhibiting lower ORAC values compared to their DPPH value are more powerful pro-oxidants than extracts with higher ORAC than DPPH, as the ORAC assay uses the extract to eliminate peroxyl radicals and protect the fluorescence probe utilised by an antioxidant chain reaction.

While the commonality between these assays lies on the basis that in vitro methods could reflect the capacity of the extract in transferring electrons or hydrogen atoms, the results reiterated the need to run multiple in vitro antioxidant assays. The use of one method has proven to not be distinctive in grains and thus multiple methods are often used.

Carotenoids required a separate extraction from TPC and antioxidant constituent extractions, as carotenoids are lipid soluble and would be underdetermined in an extraction that utilizes hexane. The extraction methods chosen were previously utilised in wheat bran (Adom et al., 2005). Recent studies have utilised similar extractions on soft wheat flours to obtain the lutein and zeaxanthin concentrations in different wheat varieties, noting a tight range of carotenoid variation in the endosperm for the analysed cultivars (Lv et al., 2012).

A previous study noted that the lutein contents of bran/germ samples were 2.4–4.9-fold higher than in the respective flour (Adom, Sorrells, & Liu, 2005). They also determined that the lutein content of whole wheat flour was similar to the bran/germ samples analysed. Therefore, our particle size samples may also vary in the whole wheat form. The production of wheat bran containing flours may offer a bioavailable lutein source.

Several carotenoids were examined due to the lipophilic nature and chemical similarities of these compounds to previously investigated tocopherols which have been to protect the kernel from oxidative damage and when consumed, thus serving as antioxidants (Adom, Sorrells, & Liu, 2003). However, additional carotenoids (α-carotene, β-cryptoxanthin) are present in wheat bran (Adom, Sorrells, & Liu, 2003, 2005). Lipid soluble tocopherols (chemically similar to carotenoids) in wheat have been noted to differ in particle size (Engelsen and Hansen, 2009), however a positive trend was not observed with the lipid soluble extracts in this study. Previous researchers have noted that not all lipid-soluble vitamins in wheat bran are available at every particle size distribution, as changes in the availability of vitamin E were seen with the reduction of particle size (Engelsen and Hansen, 2009).

5. Conclusions

For whole wheat bran at different particle size distributions the phenolic acids, anthocyanins, carotenoids and ORAC value increased as the particle size distribution decreased (greater for 200 μm than unmilled bran). Therefore, changes in particle size could affect the functional food claims with notable quantities of bran in their formulation. Further studies are needed to evaluate this in specific food products where health claims could be affected by changes in the particle size of wheat bran.

Acknowledgements

This work was made possible through the assistance of Drs. Jeffrey Gwirtz, Dan Qiu, Priyadarshini Gadgil. Support for the student’s Laureen Brewer training project was provided by the USDA National Needs Graduate Fellowship Competitive Grant No. 2008–38420–04773 from the National Institute of Food and Agriculture. Support for the student’s Jittawan Kubola research and international student exchange was provided by the Office of the Higher Education Commission, Thailand, under the program Strategic Scholarships for Frontier Research Network for the Joint Ph.D. Program Thai Doctoral degree for this research from Thailand. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable. The USDA is an equal opportunity provider and employer. This article is a US Government work and is in the public domain in the USA. This is contribution number 13-066-J from the Kansas Agricultural Experiment Station.

References


