

Extraction of Antioxidants from Wheat Bran Using Conventional Solvent and Microwave-Assisted Methods

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ABSTRACT

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Total phenolic and tocopherol contents and free radical scavenging capability of wheat bran extracted using conventional and microwave-assisted solvent extraction methods were studied. Three different solvents (methanol, acetone, and hexane) were used in the conventional solvent extraction. Methanol was the most effective solvent, producing higher extraction yield (4.86%), total phenolic compound content (241.3 μg of catechin equivalent/g of wheat bran), and free radical scavenging capability (0.042 μmol of trolox equivalent/g of wheat bran) than either acetone or hexane. However, there was no significant difference in the

total tocopherol contents (13.6–14.8 $\mu\text{g/g}$ of wheat bran) among the three different solvent extraction methods. Microwave-assisted solvent extraction using methanol significantly increased the total phenolic compound content to 467.5 and 489.5 μg of catechin equivalent; total tocopherol content to 18.7 and 19.5 μg ; and free radical scavenging capability to 0.064 and 0.072 μmol of trolox equivalent/g of wheat bran at extraction temperatures of 100 and 120°C, respectively. However, extraction yields of conventional methanol solvent and microwave-assisted methanol extractions at different temperatures were not significantly different.

Wheat bran is a rich source of various natural antioxidants that possess health benefits for humans such as preventing cardiovascular disease and certain cancers (Halliwell 1992; Truswell 2003). Phenolics, tocopherols, and fiber in wheat bran are generally believed to be primarily responsible for its positive effects on cardiovascular disease; undesirable lipid oxidation reactions in the body also contribute to these disease conditions (Moller et al 1988; Alabaster et al 1997; Andreassen et al 2001). Cholesterol oxidation could contribute to the development of a progressive thickening of the artery wall due to the accumulation of cholesterol oxidation products in low-density lipoprotein (LDL) particles after they are oxidized. Lipid oxidation reactions in the cell membrane also result in mutation of cell duplication processes and damage to the cell membrane that could cause various types of cancer (Jadhav et al 1996). Recent studies have suggested that these compounds of wheat bran exhibited significant capabilities in scavenging free radicals, chelating metal ion oxidants, and reducing lipid oxidation at different conditions (Yu et al 2002; Zhou and Yu 2004; Adom and Liu 2005). Similar to other cereal grains, wheat bran contains many different types of phenolic antioxidant compounds such as ferulic, vanillic, caffeic, coumaric, and syringic acids (Li et al 2005; Kim et al 2006) and relatively high levels of carotenoids, tocopherols, and phytosterols (Nystrom et al 2005; Zhou et al 2005).

Conventional solvent extraction at a temperature no higher than the solvent boiling point is the typical approach used in extracting antioxidants from wheat bran (Zhou and Yu 2004; Li et al 2005; Kim et al 2006). The polarities of those antioxidants in wheat bran are different, which may produce different extraction yields when solvents with different polarities are used. Low molecular weight phenolic compounds are readily extracted from cereal by methanol (Sun et al 2006). Lesser amounts of phenolic compounds are extracted by hexane due to their polar nature. Because of the existence of different types of antioxidants in the extracts obtained by different extraction solvents, the extracts may have variable capabilities in preventing lipid oxidation. Previous studies of wheat bran antioxidant used 50% acetone (Zhou and Yu 2004),

80% methanol (Kim et al 2006), and 100% methanol (Li et al 2005) as extraction solvents. Those solvents are efficient in extracting phenolic compounds or hydrophilic antioxidants but not lipophilic antioxidants such as tocopherols. In this study, three different solvents (methanol, acetone, and hexane) were used to perform conventional solvent extraction in which concentrations of total phenolic compounds, tocopherol content, and the capabilities of scavenging free radicals were compared.

Besides the conventional solvent extraction methods, microwave-assisted solvent extraction has been reported to improve the extraction efficiency of trace compounds in foods and soils. Microwave-assisted solvent extraction is accomplished either with closed vessel (under controlled pressure and temperature) or with open vessel (under atmospheric pressure) (Camel 2000; Eskilsson and Bjorklund 2000; Kornilova and Rosell-Mele 2003). The closed vessel system is mostly used in analytical-scale laboratories and is commonly known as pressurized microwave-assisted solvent extraction (Camel 2000; Eskilsson and Bjorklund 2000). The open vessel system is known as focused microwave-assisted solvent extraction where the sample is heated homogeneously by using focused microwaves in the system (Camel 2000). During microwave-assisted solvent extraction, the electromagnetic radiation is directly applied to the mixture of solvent and sample and is converted into heat. The target molecules migrate from the matrix to the solvent due to highly localized heating. Absorption of microwave energy depends on the nature of solvent and sample matrix. Polar molecules and ionic solutions will strongly absorb microwave energy. In most cases, the solvent selected for extraction, such as methanol or isopropanol, has a high dielectric constant and will strongly absorb microwave energy. However, in extracting some thermolabile components, the extraction temperature is particularly controlled to avoid the degradation of involved components. Recent studies demonstrated that the microwave-assisted extraction method increased the recovery of trace residues in vegetables, fruits, coffee, tea, and beans (Ganzler et al 1986; Negeri et al 2000; Falqui-Cao et al 2001; Diagne et al 2002; Pan et al 2003; Singh et al 2004). Antioxidants and antioxidant activity of asparagus also increased after microwave-circulated water treatment (Sun et al 2007). In this study, the solvent with the highest extraction efficiency in the conventional solvent extraction study was used in microwave-assisted extraction media. The concentrations of total phenolic compounds, tocopherol content, and free radical scavenging capabilities of wheat bran at different microwave-assisted extraction temperatures were evaluated. The information of this study would be helpful in the development and utilization of wheat bran as a food antioxidant or as an antioxidant nutritional supplement.

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MATERIALS AND METHODS

Chemicals and Materials

Hard white wheat bran was a gift from General Mills Company Bell Institute of Health and Nutrition (Minneapolis, MN). The bran was ground to pass through a No. 40 sieve (0.425 mm) and stored at 4°C before use. The 2,2'-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (trolox), Folin-Ciocalteu reagent, acetic acid, and α -, γ -, and δ -tocopherols were purchased from Sigma-Aldrich (St. Louis, MO). Methanol, acetone, hexane, and ethyl acetate were HPLC-grade and purchased from Fisher Scientific (Springfield, NJ).

Conventional Solvent Extraction

Methanol (bp 64.7°C), acetone (bp 56.0°C), and hexane (bp 69.0°C) were the solvents used for the conventional solvent extraction. Each extraction (methanol, acetone, and hexane) was done in triplicate. Wheat bran (10 g) was transferred into test tubes (25 × 150 mm) to which 40 mL of each solvent was added and vortexed for 30 sec. The test tubes were capped and placed in a 60°C water bath for 20 min. These test tubes were vortexed twice during the incubation. Then the solvent layer in each tube was separated by centrifuging at 2,000 × *g* for 15 min. The solvent supernatant was transferred to a previously weighed clean test tube. The residue was again mixed with 20 mL of the same solvent. The supernatant was separated as previously described and combined with the previous supernatant. The tube containing supernatant was then placed in a vacuum centrifuge evaporator (CentriVap Mobile System; Lab-conco, Kansas City, MO) to remove solvent. The dried extract in the test tube was weighed to measure the extraction yield of the samples, then stored at -20°C before further testing. The extraction yield (%) was calculated as (g of extract/g of fresh wheat bran) × 100. The water content of the wheat bran was 12.76 ± 0.05% (*n* = 4).

Microwave-Assisted Solvent Extraction

Wheat bran (10 g) was transferred into each of the three Teflon vessels of a microwave extraction system (Ethos E, Milestone, Monroe, CT). Only methanol was used as a solvent and heated over its boiling point (64.7°C) during microwave-assisted extraction. The Teflon vessels were covered with a polymer material that can resist high inside pressure generated when the extraction temperatures are higher than the solvent boiling point. Methanol (40 mL) with a magnetic stirring rod was added to each vessel. The vessels were sealed and placed inside the microwave-extraction system. The stirring rod was rotated by an electrical magnetic plate under each vessel to keep the inside temperature even. The microwave-extraction system was programmed to increase to the extraction temperature with an energy level of 800W and held at that temperature for 20 min with an energy level of 500W. Four extraction temperatures (60, 80, 100, and 120°C) were applied. After 30 min of cooling to room temperature, the vessels were unsealed and the contents were quantitatively transferred to centrifuge tubes in which supernatant was prepared as described for the conventional solvent extraction. The residue was mixed with 20 mL of the same solvent and vortexed again. The solvent supernatant was separated by centrifugation at 2,000 × *g* and combined with the previous supernatant. The supernatant was dried as described above. The dried extract in the test tube was weighed to measure extraction yield of the samples. It was stored at -20°C before use. The extraction yield (%) was calculated as above.

Determination of Total Phenolic Compounds

The total phenolic compound content of wheat bran extract was determined using Folin-Ciocalteu reagent (Velioglu et al 1998) that was diluted 10× with deionized water. Dried wheat bran extract (0.020 g) was redissolved in 1 mL of methanol, and 0.1 mL of this bran extract solution was mixed with 0.75 mL of the diluted

Folin-Ciocalteu reagent. The reaction solution was left at room temperature for 5 min. Then 0.75 mL of sodium bicarbonate solution (60 g/L) was added to the reaction solution. The mixture was incubated at room temperature for 90 min and filtered through a 0.45- μ m syringe filter (Pall Corporation, Ann Arbor, MI). The absorbance of the solution was determined at 750 nm using a spectrophotometer (UV-Visible SpectraMax Plus³⁸⁴, Molecular Devices, Sunnyvale, CA). The test for each extract was duplicated. The averaged absorbance was used in calculation. Catechin was used to prepare a standard curve. The total phenolic compound content was expressed as μ g of catechin equivalent/g of wheat bran.

Determination of Total Tocopherol Content Using HPLC

The HPLC system consisted of Waters (Milford, MA) 510 pumps, a 715 Ultra WISP injector, and 470 fluorescence detector. Chromatograms were recorded and processed using Waters Millennium chromatography software. Samples were injected into a column (25 cm × 4.6 mm diameter) of 5- μ m Supelcosil LC-Si (Supelco, Bellefonte, PA). The column was preceded by a 5 cm × 4.6 mm, i.d., guard column packed with 40- μ m pellicular silica. The mobile phase consisted of 0.5% ethyl acetate and 0.5% acetic acid in hexane at a flow rate of 1.5 mL/min. The fluorescence detector was set at 290 nm excitation and 330 emission. The dried extract (0.200 g) was dissolved in 10 mL of hexane and vortexed. Solution (100 μ L) was injected into the HPLC system. The tocopherol concentration was calculated based on the standard curve. The total tocopherol content was calculated by summing each tocopherol concentration and converting to μ g/g of wheat bran.

Determination of Antioxidant Activity Using DPPH Radical Scavenging Method

The wheat bran extract solution for the DPPH test was prepared by redissolving 0.200 g of each dried extract in 10 mL of methanol. The concentration of DPPH solution was 0.025 g in 1,000 mL of methanol. The DPPH solution (2 mL) was mixed with 40, 80, and 120 μ L of the bran extract/methanol solution and transferred to a cuvette. The reaction solution was monitored at 515 nm for 30 min at room temperature using the UV-Visible SpectraMax Plus³⁸⁴ spectrophotometer. The inhibition percentage of the absorbance of the DPPH solution was calculated using the equation

$$\text{Inhibition\%} = \{(\text{Abs}_{t=0\text{min}} - \text{Abs}_{t=30\text{min}}) / \text{Abs}_{t=0\text{min}}\} \times 100$$

where $\text{Abs}_{t=0\text{min}}$ was absorbance of DPPH at zero time and $\text{Abs}_{t=30\text{min}}$ was absorbance of DPPH after 30 min of incubation.

The inhibition percentage of the absorbance of DPPH was plotted against each quantity of bran extract solution to obtain a regression line. Trolox (0.5 mM) was dissolved in methanol and used as a standard to convert the inhibition capability of the bran extract solution to the trolox equivalent. The ratio between the slopes of the regression lines of the bran extraction solution and the trolox solution was defined as μ mol of trolox equivalent antioxidant activity.

Statistical Analysis

The mean values and standard deviations of the extraction yield, total phenolic compounds, tocopherol contents, and the scavenging DPPH capability were analyzed using one-way ANOVA with multiple comparisons by Fisher's least significant difference test to determine significant difference at *P* < 0.05 (Zar 1996).

RESULTS AND DISCUSSION

Extraction Yields of Wheat Bran Obtained by Conventional Solvent and Microwave-Assisted Solvent Extractions

The extraction yields of wheat bran obtained by different solvent and microwave-assisted methanol extractions are shown in Fig. 1. The yields of acetone and hexane extraction were \approx 2.40%

and were not significantly different from each other. These extraction yields were close to 2.21%, which was the raw wheat bran fat content using the petroleum-ether extraction method reported by Wang et al (1993). However, both were approximately half that of the methanol extraction, which was 4.86% of raw wheat bran. The extraction yields of the three solvent extractions were not significantly increased when extraction time was increased to >20 min. The solvent polarity from high to low is methanol > acetone > and hexane. Therefore, it is likely that the chemical compositions of the three extracts could be widely different (Sun et al 2006). Hexane and acetone solvent would extract more of the less polar triglycerides, phytosterols, and phospholipids of wheat bran. Wang et al (1993) reported that the content of extractable fiber and protein were higher in the raw bran than in the whole wheat. Thus, methanol solvent may extract not only lipids and small molecules of polar compounds but also some large molecules of polar compounds such as alcohol-soluble proteins and carbohydrates from wheat bran.

The extraction yields of microwave-assisted solvent extractions with methanol had a range of 4.71–5.01%, regardless of the extraction temperatures. The microwave-assisted extraction did not significantly increase the extraction yield compared with the conventional methanol solvent extraction. The major advantage of microwave-assisted extraction is to use microwave energy to heat the solvent and sample to increase the mass transfer rate of solutes from the sample matrix into the solvent (Sporring et al 2005). In this study, the particle size of wheat bran powder was small, which may have precluded the mass transfer advantage obtained with microwave-assisted extraction. Consequently, extraction yield was not greatly increased in the microwave-assisted extraction process.

Total Phenolic Compounds and Tocopherol Contents of Wheat Bran Extracted by Different Solvent and Microwave-Assisted Methanol Extractions

Figure 2 shows the total phenolic compound contents of wheat bran extracted using the different solvent and microwave-assisted methanol extractions. The order of total phenolic compound content from low to high was obtained from the hexane, acetone, and methanol extractions with 59.1, 88.0, and 241.3 µg of catechin equivalent/g of wheat bran, respectively. The result is similar to that reported by Sun et al (2006), where methanol was most effective in extracting phenolic compounds from oat bran. In that study, the phenolic compound content extracted by methanol was ≈3× higher than that extracted by acetone and 4× higher than that extracted by hexane. The microwave-assisted methanol extraction significantly increased total phenolic compound content extracted from wheat bran when the extraction temperature was >80°C. The

microwave-assisted extraction at 100 or 120°C was able to extract the highest level of phenolic compounds from wheat bran among all studied extraction conditions. At these temperatures, >467.5 µg of catechin equivalent/g of wheat bran was observed, which was ≈2× higher than the highest level produced by the conventional methanol extraction. Extraction recovery of trace residues in vegetables, fruits, coffee, and beans by microwave-assisted extraction were higher than those by the solvent extraction as reported in several studies (Ganzler et al 1986; Negeri et al 2000; Falqui-Cao et al 2001; Diagne et al 2002; Pan et al 2003; Singh et al 2004). For example, using microwave extraction, the recoveries of the three trace pesticides from spiked vegetable samples were increased ≤2× compared with the traditional solvent extraction (Singh et al 2004). Wheat bran has extractable and bound phenolic compounds (Kim et al 2006; Liyana-Pathirana and Shahidi 2006). In these studies, the total phenolic compound content increased after wheat bran was treated with acid or alkaline solution to hydrolyze bound phenolic compounds. In our present study, the increased extraction temperature and microwave energy may break down or increase hydrolysis of the bonds of some bound phenolic compounds and cause them to become extractable phenolic compounds. A similar finding was observed by Sun et al (2007), where the rutin level of asparagus was increased with the microwave energy treatment.

Total tocopherol contents in wheat bran extract obtained by different solvent and microwave-assisted methanol extractions are shown in Fig. 3. The total tocopherol contents of the three different solvent extractions and microwave-assisted methanol extractions at 60 and 80°C were not significantly different and had a range of 13.6–15.8 µg/g of bran.

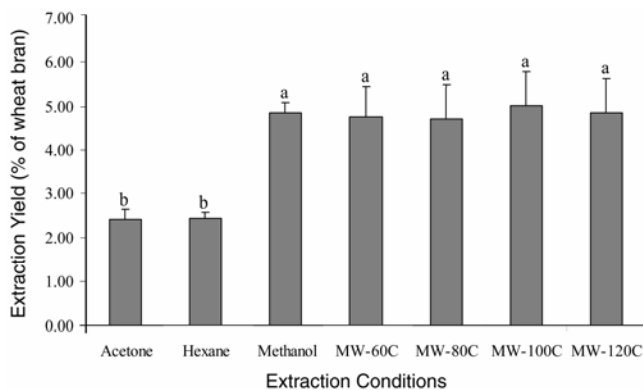


Fig. 1. Extraction yield (% of wheat bran) obtained by different solvent and microwave-assisted methanol extractions at different temperatures (60, 80, 100, and 120°C). MW, microwave-assisted methanol extraction. Bars topped with different letters are significantly different ($P < 0.05$).

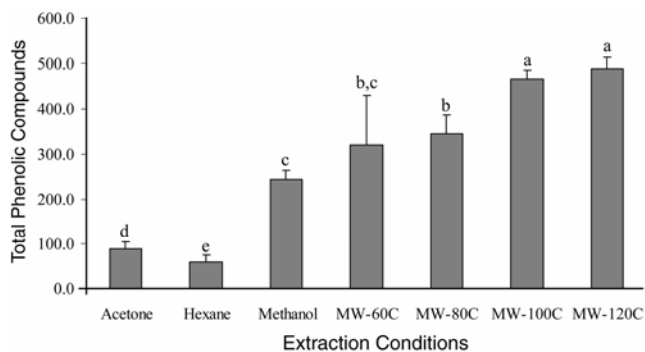


Fig. 2. Total phenolic compounds (catechin equivalent µg/g of wheat bran) from wheat bran extracted by different solvent and microwave-assisted methanol extractions at different temperatures (60, 80, 100, and 120°C). MW, microwave-assisted methanol extraction. Bars topped with different letters are significantly different ($P < 0.05$).

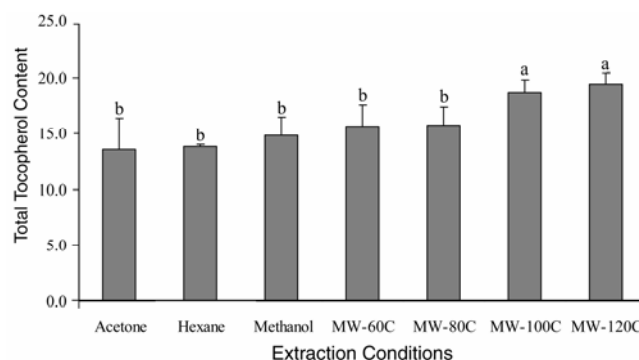


Fig. 3. Total tocopherol content (µg/g of wheat bran) from wheat bran extracted by different solvent and microwave-assisted methanol extractions at different temperatures (60, 80, 100, and 120°C). MW, microwave-assisted methanol extraction. Bars topped with different letters are significantly different ($P < 0.05$).

The reported range of total tocopherol contents in different cultivars of wheat bran was 7.98–12.48 $\mu\text{g/g}$, which was obtained using 50% acetone solvent extraction (Zhou et al 2005). When compared with the results for extracting hydrophilic phenolic compounds (Fig. 2), the three solvents in this study did not demonstrate differences in efficiency of extracting the lipophilic tocopherols (Fig. 3). We have found that the extraction capabilities of a single solvent for different tocopherol homologues are different (Xu 2002). Hexane extracted a greater amount of α -tocopherol, while relatively higher polar solvents such as ethyl acetate extracted more of the other tocopherols (β , γ , δ). However, when the microwave-assisted extraction temperature was increased to 100 and 120°C, the total tocopherol contents of bran were increased to 18.7 and 19.5 $\mu\text{g/g}$, respectively, which was significantly higher than all other extraction conditions. Similar to the extraction of phenolic compounds (Fig. 2), the higher extraction temperature and microwave energy may free up esterified tocopherols into a more extractable form.

Free Radical Quenching Capability of Wheat Bran Extracts by Different Solvent and Microwave-Assisted Methanol Extractions

The results of free radical quenching capabilities of wheat bran obtained by using different solvent and microwave-assisted methanol extractions are shown in Fig. 4. The order of the free radical quenching capabilities from high to low by different solvent extractions was methanol > acetone > and hexane. This is in agreement with the results obtained by Sun et al (2006) using these three solvents for extracting antioxidants from an oat bran sample. Also, the free radical quenching capabilities of wheat bran extract was increased with increasing temperature of microwave-assisted extraction. At a microwave-assisted extraction temperature of 120°C, the capability (0.072 μmol of trolox equivalent/g of wheat bran) was highest among all extractions. The DPPH test has been used for evaluating antioxidant activity of wheat bran extract in many studies (Zhou and Yu 2004; Adom and Liu 2005; Li et al 2005; Kim et al 2006). The extracts from bran from various wheat genotypes provided greater scavenging DPPH capability than the whole wheat meal (Li et al 2005). Several studies suggested that the total phenolic content may have a positive correlation with antioxidant activity (Velioglu et al 1998; Emmons et al 1999). Hydrophilic phenolic antioxidants contributed >98% of the total antioxidant activity of grain samples (Adom and Liu 2005). In our study, because all extracts contained similar quantities of lipophilic tocopherol antioxidants (Fig. 3), differences of the total hydrophilic phenolic compound content (Fig. 2) may be responsible for the differences in the DPPH scavenging capability (Fig. 4). This suggests that important antioxidants in wheat bran are polar phenolic

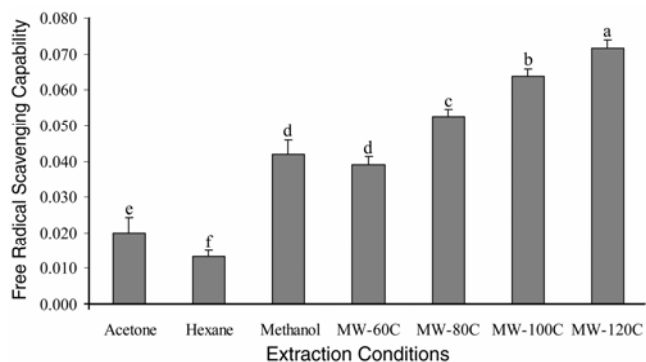


Fig. 4. Capabilities of scavenging DPPH free radicals (trolox equivalent $\mu\text{mol/g}$ of wheat bran) from wheat bran extracted by different solvent and microwave-assisted methanol extractions at different temperatures (60, 80, 100, and 120°C). MW, microwave assisted methanol extraction. Bars topped with different letters are significantly different ($P < 0.05$).

compounds and are likely to be more extractable in methanol than in either acetone or hexane, especially at the higher temperatures and without causing the thermal degradation of those compounds. The higher temperature generated in microwave extraction could help release more free polyphenolic compounds such as phytochemicals and anthocyanin (Pan et al 2003). Those compounds in cereals and grains also were reported to have a greater capability in scavenging free radicals (Xu et al 2005).

CONCLUSIONS

In the conventional solvent extractions, methanol showed the greatest capability in extracting phenolic antioxidants and inhibiting the free radicals produced by DPPH among the three solvents (methanol, acetone, and hexane). Microwave-assisted extraction with methanol as a media increased the extraction efficiencies for both hydrophilic and lipophilic antioxidants in the wheat bran extract at 100 and 120°C, respectively. The significantly higher hydrophilic antioxidants extracted by the microwave-assisted extraction resulted in a higher capability in scavenging free radicals. Microwave-assisted methanol extraction is therefore an efficient method for extracting antioxidants from wheat bran compared with conventional solvent extraction methods.

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