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Total Phenolic Content of Cereal Brans using Conventional and Microwave Assisted Extraction

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ABSTRACT

Total phenolic content of cereal brans (wheat, rice, oat) singly and in combination (wheat: rice: oat:: 2:1.5:1.5) extracted using conventional and microwave-assisted solvent extraction (2, 3.5 and 5 min) methods were studied. Three different solvents (methanol, acetone and hexane) were used in the conventional solvent extraction using three different temperatures (50, 60 and 70°C). Methanol at 60°C was the most effective solvent, producing higher total phenolic compound content in all types brans singly and in combination than either acetone or hexane. Microwave-assisted solvent extraction significantly increased the total phenolic compound content in solvents used at 2450 MHZ for 3.5 min. Maximum total phenolic content was recorded in methanolic extracts using microwave energy at 2450 MHZ for 3.5 min. However, total phenolic content of conventional and microwave-assisted extractions at different temperatures and different time durations, respectively were significantly different ($p \leq 0.05$). The mean total phenolic content of brans ranged from 1.24-2.87 and 2.20-4.09 mg GAE g^{-1} by conventional and microwave assisted extraction, respectively. Among different solvents methanolic extract recorded maximum mean total phenolic content in both types of extraction systems i.e., 2.83±0.17 and 4.50±0.25 mg GAE g^{-1} in conventional and microwave extraction, respectively.

Key words: Total phenolic content, cereal brans, conventional, microwave extraction

INTRODUCTION

Cereal brans, the by-products obtained in large amounts in grain milling industry, considered as inedible material for humans, is mostly used as animal feed. However, brans are concentrated source of dietary fibre and other nutrients (proteins, B-vitamins and minerals). Brans are generally composed mainly of insoluble cellulose and hemicellulose, with only about 5% soluble fibre and has little hypercholesterolemia effect (Kay and Truswell, 1980). Antioxidants are concentrated in bran fractions, although the endosperm has significant activity. It is not easy to determine how much of the bound or insoluble antioxidants is measured by a given assay. The level of antioxidant activity in blood increases after the consumption of foods high in antioxidants, If the bran of whole grains is essentially intact when it is consumed, the cell contents will be much less available for absorption. Whole grain antioxidants can act as free radical scavengers (Spiller, 2001). 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, 1996; Truswell, 2002). Phenolics, tocopherols and fiber are generally believed to be primarily responsible for wheat bran's positive effects on cardiovascular disease; undesirable lipid oxidation reactions in the body also contribute

to these disease conditions (Alabaster *et al.*, 1997; Andersen *et al.*, 2001; Moller *et al.*, 1988). The antioxidant compounds in rice bran have purported health benefits as well as antioxidant characteristics for improving the storage stability of foods. Several studies have reported the effects of rice bran on metabolic activities including reduced plasma cholesterol in laboratory animals and humans (Yoshino *et al.*, 1989; Qureshi *et al.*, 1991; Hegsted and Windhauser, 1993). Oryzanol has been studied for its ability to reduce cholesterol absorption (Rong *et al.*, 1997). Komiyama *et al.* (1992) and Nesaretnam *et al.* (1998) reported anticancer activity associated with tocotrienols. Thus, rice bran is viewed as a potential source of these high-value antioxidants for use as additives in foods, pharmaceuticals and cosmetics. Lipid oxidation is believed to be the cause of most off-flavors in food products. Since oat bran naturally contains high levels of antioxidants the concentration of these antioxidants could be added to food products to lower the rate of lipid oxidation and potentially add additional health benefits. The applied use would be for product developers to use healthy but easily oxidized lipids when developing new food products and prevent oxidation by adding the antioxidant-rich oat bran extract.

Traditional Solvent Extractions (TSE) have been widely used with debatable accuracy for attaining total antioxidant capacities in food products. The difficulty scientists have had is keeping the assays consistent. The variations are many; antioxidants can be extracted using different solvents such as ethanol, acetone, methanol and hexanol and these solvents can each be employed with different ratios of dilution altering results. Microwave-assisted Solvent Extraction (MASE) is a relatively new extraction method for scientists studying antioxidant capacity in food. One prevalent application in analytical chemistry is the use of microwave energy as auxiliary energy. However, little documentation can be found utilizing a microwave for extracting antioxidants and phenolics from food products.

Several studies on antioxidant properties of cereal brans have been carried out, the data is still deficient. Our aim was to study some cereal brans for total phenolic content for use as sources (i) in human consumption preventing disease and promoting health and (ii) in food processing, preserving oxidative alterations.

MATERIALS AND METHODS

Materials

Raw materials used in the investigation: Commercial wheat flour, wheat bran, rice bran and oat bran (Baggry's India Ltd., New Delhi, India) were purchased from local market. Wheat bran was collected from Ludhiana Flour Mill, Ludhiana, India. Rice bran was purchased from 'Ricela Health Foods Ltd.', Dhuri, Punjab, India.

Preparation of samples: Different cereal brans procured were packed for further processing.

Physico-chemical composition of raw materials: Physico-chemical characteristics (Moisture content, crude protein, ash, crude fibre and carbohydrate) of wheat bran, rice bran and oat bran were determined using standard methods (AACC, 2000).

Assessment conditions: Two extraction methods were followed for potential extraction of antioxidants. Following variables were used to extract the antioxidants from cereal Bran.

Traditional method:

Temperature : 50, 60, 70°C
Solvent : Acetone, Hexane and Methanol

Acetone, hexane and methanol were the solvents used for the traditional solvent extraction. Ten grams of ground fine bran weighed using an aluminum container and transferred into a test tube (25×150 mm). Each solvent (40 mL) was added in a test tube and vortexed to mix with the bran sample well. The test tubes were capped and placed in a water bath at different temperatures (50, 60 and 70°C) for 20 min. These test tubes were vortexed twice during the incubation. Then, the solvent layer from each test tube was separated by centrifugation at 7000 rpm for 10 min. The solvent supernatant was transferred to clean, previously weighed and labeled test tubes. The residue was mixed with 20 mL of the same solvent again and vortexed. The solvent supernatant was combined with the previous one. The dried extract in the test tube was weighed to measure the extraction yield of the samples. All samples were placed in a refrigerator prior to testing.

Microwave extraction: Ten grams bran was weighed using a clean aluminum container and transferred conical flasks. The conical flasks were covered in such a way so as to resist high inside pressure generated when extraction temperatures are higher than the used solvent's boiling point. Each solvent (40 mL) was added and stirred with glass rod to mix with the bran sample well. Then, the Microwave Extraction System was programmed to increase to the extraction time with a maximum energy at 2450 MHZ. Three extraction times, 2, 3.5 and 5 min, were applied to perform the microwave-assisted solvent extraction. After ten-minute cool down period, the vflasks were unsealed and transferred to each corresponding centrifuge tube. These tubes were centrifuged at 7000 rpms for 10 min to separate the supernatant and residue. The solvent supernatant was transferred to a clean test tube that had been previously weighed. The residues were mixed with 20 mL of the same solvent again and vortexed. The solvent supernatant was separated by the centrifugation and combined with the previous one. The dried extract in the test tube was weighed to measure the extraction yield of the samples. All samples were placed in a refrigerator prior to testing.

Total phenolic assay (Prior *et al.*, 2005)
Chemicals:

- Folin-Ciocalteu reagent
- 7% Na_2CO_3 solution

Estimation: The total phenolic content of cereal brans were determined by using the Folin-Ciocalteu assay. An aliquot (1 mL) of extracts or standard solution of gallic acid (20, 40, 60, 80 and 100 mg L^{-1}) was added to 25 mL volumetric flask, containing 9 mL of distilled water (dd H_2O). A reagent blank using dd H_2O was prepared. One milliliter of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken After 5 min, 10 mL of 7% Na_2CO_3 solution was added to the mixture. The solution was diluted to volume (25 mL) with dd H_2O and mixed. After incubation for 90 min at room temperature, the absorbance against prepared reagent blank was determined at 750 nm with spectronic-20. Total phenolic content was expressed as mg gallic acid equivalents (GAE)/100 g weight.

Statistical analysis: The mean values and standard error of total phenolic compounds were analyzed by Minitab Software with Completely Randomised Design (CRD) in factorial experiment using three replications. The means were compared with Duncans multiple range test.

RESULTS AND DISCUSSION

Proximate composition of raw materials: Data embodied in Table 1 represented the proximate composition of cereal brans (wheat bran, rice bran and oat bran). All the ingredients possessed variation statistically with respect to their chemical constituents.

Among the various cereal brans, oat bran had the highest value for crude proteins (15.03%) whereas wheat bran had the least (9.6%). The ash content of cereal brans ranged between 1.45-6.72%. Rice bran was higher in ash content (6.72%), whereas oat bran had minimum ash content (1.4%). Percent fat content of all the raw material used ranged from 4.07 to 19.31%. Among various brans, rice bran had the maximum value for fat (19.3%) followed by oat bran (10.59%). Wheat bran (4.06%) had lowest fat percentage of crude fat. Wheat bran and rice bran contained 33.4 and 38.9% dietary fiber, respectively. Oat bran had a least value for dietary fiber that is 14.0%. Rice bran had highest amounts of crude fiber (11.48%). While as, wheat bran contained 7.75% crude fiber and oat bran had least value of crude fiber (3.31%) among different cereal brans. Maximum carbohydrate was found in wheat bran (60.52%) followed by oat bran (55.62%). Rice bran contained lowest carbohydrates 36.63%. The extent of carbohydrate in any food product has inverse relation with fiber present.

Similar results regarding proximate composition of brans were reported in several studies (Singh *et al*., 1995; Slavin and Lampe, 1992; Bhatty, 1993; De-Dalahaye *et al*., 2002).

Total phenolic content of cereal brans extracted by conventional method of solvent extraction: Total phenolic content (mg GAE/g) of cereals extracted by conventional extraction method is presented in Table 2. Total phenolic content of brans and their combination differed significantly ($p \leq 0.05$). The order of the total phenolic content in various brans from low to high was oat (1.24 mg GAE g^{-1}), wheat (2.57 mg GAE g^{-1}) and rice (2.87 mg GAE g^{-1}). Further bran in combination (wheat:rice:oat:: 2:1.5:1.5) reported total phenolic content of 2.26 mg GAE g^{-1}. Dykes and Rooney (2007) reported similar trend of total phenolic content while studying pehnolic compound in cereal grains. Herrmann (1989) reported that total phenolic compounds in rice bran varied significantly. The difference in total phenolic content among various cereal brans may be due to the difference in heat labile nature of cereal bran.

The effect of different solvents on the extraction of total phenolic compounds varied significantly. The mean values for total phenolic compound were 1.60, 2.27 and 2.83 mg GAE g^{-1} by using different solvents hexane, acetone and methanol, respectively. The results are similar to that reported by Sun *et al*. (2006), they found methanol as most effective solvent in extracting

Table 1: Proximate composition* of raw materials

Raw materials	Crude protein (%)	Ash (%)	Crude fat (%)	Dietary fiber (%)	Crude fiber (%)	Carbohydrates (%)
Wheat bran	9.60^{a}	4.06^{c}	4.07^{b}	33.4^{c}	7.75^{c}	60.52^{c}
Rice bran	11.86^{c}	6.72^{d}	19.31^{d}	38.9^{d}	11.48^{d}	36.63^{a}
Oat bran	15.03^{d}	1.45^{b}	10.59^{c}	14.0^{b}	3.31^{b}	55.62^{b}

*Expressed at 14% moisture basis. Percentage mean values with different superscript alphabet in the same column are significantly different at $p \leq 0.05$

Table 2: Total phenolic content (mg GAE g^{-1}) of cereal brans extracted by conventional extraction

	Type of bran				
Extraction conditions solvent/temp	Wheat	Rice	Oat	In combination	Mean
Acetone					
50°C	2.03	1.72	0.98	1.62	1.59
60°C	3.34	3.97	1.15	2.87	2.83
70°C	2.75	3.60	0.81	2.42	2.39
Mean	2.70	3.09	0.98	2.30	2.27
Hexane					
50°C	1.41	1.28	1.08	1.27	1.26
60°C	2.06	2.30	1.98	2.11	2.11
70°C	1.71	1.51	1.19	1.45	1.43
Mean	1.72	1.69	1.37	1.61	1.60
Methanol					
50°C	2.58	3.38	1.19	2.40	2.38
60°C	4.17	4.18	1.75	3.45	3.38
70°C	3.13	3.86	1.17	2.76	2.73
Mean	2.97	3.81	1.37	2.87	2.83

Bran	Values	Solvent	Values	Temperature (°C)	Values
Wheat	2.57±0.16^{c}	Acetone	2.27±0.17^{b}	50	1.74±0.12^{a}
Rice	2.87±0.21^{d}	Hexane	1.60±0.07^{a}	60	2.78±0.17^{c}
Oat	1.24±0.07^{a}	Methanol	2.83±0.17^{c}	70	2.19±0.17^{b}
In combination	2.26±0.13^{b}				

Mean values with different superscript alphabet in the same column are significantly different at $p \leq 0.05$

phenolic compounds from oat bran. Oufnac *et al.* (2007) also reported similar results while working on extraction of antioxidant from wheat bran using conventional solvents.

It is also evident from the table that with increase in solvent extraction temperature, there was significant increase in total phenolic content in cereal brans. Maximum total phenolic contents were obtained at 60°C (2.78 mg GAE g^{-1}) while as minimum at 50°C (1.74 mg GAE g^{-1}). Oufnac *et al.* (2007) reported that with rise in extraction temperature more phenolic compounds are released. Earlier research has shown that higher temperature during extraction has a tendency to increase antioxidant activity (Brand-Williams *et al.*, 1995). Increased extraction temperature may breakdown or increase hydrolysis of the bonds of some bound phenolic compounds and cause them to become extractable phenolic compounds. Similar fact was observed by Sun *et al.* (2007) while extracting total phenols from asparagus.

These results are in agreement with previous reports which also reported that antioxidants including phenolics are concentrated in the aleurone fraction of bran (Onyeneho and Hettiarachchy, 1992; Fulcher *et al.*, 1972).

All the interactions among cereal barns, solvents and temperature had significant effect on total phenolic content. Maximum total phenolic content (4.18 mg GAE g^{-1}) was recorded in rice bran at 60°C when methanol was used as solvent.

Total phenolic content of cereal brans extracted by microwave assisted solvent extraction: Effect of microwave assisted solvent extraction and type of bran is depicted in Table 3. Statistically significant variation was observed among various cereal brans in terms of total phenolic content obtained by microwave assisted extraction. Among various cereal brans rice bran

Table 3: Total phenolic content (mg GAE g^{-1}) of cereal brans extracted by microwave extraction

Extraction conditions	Type of bran				
solvent/time	Wheat	Rice	Oat	In combination	Mean
Acetone					
2 min	3.04	2.22	2.01	2.48	2.44
3.5 min	4.51	4.57	2.23	3.84	3.54
5 min	3.82	4.30	1.70	3.33	3.29
Mean	3.79	3.70	1.98	2.88	3.09
Hexane					
2 min	1.64	1.78	1.58	1.67	1.67
3.5 min	2.66	2.90	2.53	2.69	2.69
5 min	2.41	2.21	1.76	2.15	2.13
Mean	2.24	2.30	1.96	2.17	2.16
Methanol					
2 min	3.88	5.08	2.32	3.89	3.79
3.5 min	4.78	7.94	3.45	5.64	5.45
5 min	4.56	5.84	2.29	4.39	4.27
Mean	4.40	6.28	2.68	4.64	4.50

Bran	Values	Solvent	Values	Time (min)	Values
Wheat	3.47 ± 0.36^{c}	Acetone	3.09 ± 0.16^{b}	2.0	2.63 ± 0.18^{a}
Rice	4.09 ± 0.22^{d}	Hexane	2.16 ± 0.08^{a}	3.5	3.89 ± 0.27^{c}
Oat	2.20 ± 0.10^{a}	Methanol	4.50 ± 0.25^{c}	5.0	3.23 ± 0.22^{b}
In combination	3.23 ± 0.23^{b}				

Mean values with different superscript alphabet in the same column are significantly different at $p \leq 0.05$

reported maximum total phenolic content (4.09 mg GAE g^{-1}) followed by wheat bran (3.47 mg GAE g^{-1}). Cereal brans in combination had 3.23 mg GAE g^{-1} total phenolic content while as minimum total phenol content was recorded in oat bran (2.20 mg GAE g^{-1}). A significant difference was assessed in total phenolic content of brans extracted by using variable solvents. Maximum total phenolic content was observed in methanolic extracts (4.50 mg GAE g^{-1}) followed by acetone (3.09 mg GAE g^{-1}) and hexane (2.16 mg GAE g^{-1}). Sun *et al.* (2006) reported that phenolic content extracted by methanol was three times higher than acetone and four times higher than hexane.

Microwave treatment for extraction had significant effect on total phenolic content of cereal brans. With increase in exposure time there was significant increase in total phenolic content in brans. Maximum total phenolic content (3.89 mg GAE g^{-1}) was recorded at microwave heating for 3.5 min, reduced time (2 min) and higher time (5 min) showed less total phenolic content in brans when exposed to microwave for extraction. Emmons *et al.* (1999) reported that catechin equivalency for phenolic content of oat from methanol extractions ranged from 9.8-44.4 mg kg^{-1}.

All interaction among type of brans, type of solvent and time of exposure had statistically significant ($p \leq 0.05$) effect on total phenolic content. Maximum total phenolic content under microwave assisted extraction was obtained in rice bran at 3.5 min microwave exposure using methanol as extracting medium. Oufnac *et al.* (2007) reported that microwave assisted methanol extraction significantly increased total phenolic content extracted from wheat bran when extraction temperature was greater than 80°C. The higher extraction temperature and microwave energy may free up polyphenols into a more extractable form.

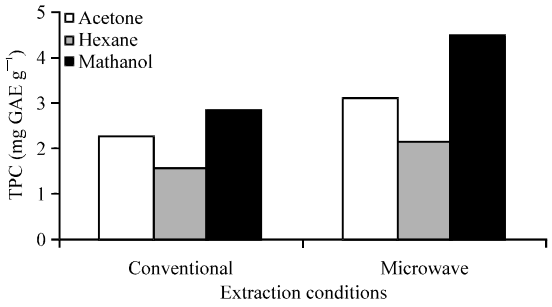

Fig. 1: Total phenolic contents (mg GAE g^{-1}) of cereal brans

Comparison between traditional and microwave assisted extraction: Figure 1 illustrates the comparison between two extraction methods. Microwave assisted extraction offered higher total phenolic content when compared to conventional extraction and similar results were reported by Holliday (2006). Microwave energy may breakdown or increase hydrolysis of the bonds of some phenolic compounds and make them to become extractable phenolic compounds (Oufnac *et al.*, 2007). Higher extraction recovery of trace residue in foods, vegetables, fruits, coffee and bean by microwave assisted extraction than solvent extraction was reported in several studies (Diange *et al.*, 2002; Pan *et al.*, 2003; Singh *et al.*, 2004).

CONCLUSION

In the conventional solvent extractions, methanol showed the greatest capability in extracting phenolic antioxidants among the three solvents (methanol, acetone and hexane). Microwave-assisted extraction with methanol as media increased extraction rate of total phenolic compounds in all types of cereal brans. Rice bran recorded maximum total phenolic content under both types of extraction system. Microwave-assisted methanol extraction is an efficient method for extracting total phenolic compounds from cereal brans compared with conventional solvent extraction methods.

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