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Original Article

Chemical analysis and antioxidant properties of foxtail millet bran extracts

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Abstract

The aim of this research was to extract phenolic compounds and assess the antioxidant potential of defatted foxtail millet bran (DFMB) extracts. Distilled water, 30%, 50%, and 70% ethanol were used as solvent for extraction. The proximate composition, fatty acid analysis, total phenolic content and extraction yield were carried out. The antioxidant efficacies of DFMB extract were tested using 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), superoxide radical and reducing power. The highest inhibitory concentration (IC_{50}) values scavenging on DPPH, ABTS and superoxide radicals were in the range of 0.131±0.002, 0.795±0.003, and 0.158±0.009 mg/ml, for distilled water, and 30 and 50% ethanol extracts respectively. The reducing power increased with increase of sample concentration. Total phenolic content ranged from 21.49±3.26 to 29.39±1.36 gallic acid equivalent (GAE)/100 g extract. The phenolic content and antioxidant activity in DFMB extracts were significantly correlated ($p<0.05$). These results indicated that 50% ethanol extract from DFMB appeared to be the most promoting phenolic compound with substantial antioxidant activity.

Keywords: extraction, antioxidant activity, foxtail millet bran, total phenolic content

1. Introduction

Cereals are the staple diet of most of the world's population. Foxtail millet (*Setaria italic*a) is one of the most important food crops of the semiarid tropics, originated from China, and is now planted all over the world (En *et al.,* 2008; Amadou *et al.,* 2011). It plays a very important role in the agriculture and food of many developing countries because of its ability to grow under adverse heat and limited rainfall conditions. It was reported that foxtail millet has many nutritious and medical functions. Foxtail millet is non-glutinous, like buckwheat and quinoa, and it is not an acid forming food, so it is soothing and easy to digest. In fact, it is considered to be one of the least allergenic and most digestible grains available and it is a warming grain (Prashant *et al.,* 2005; Xue *et al.,* 2008). The millet bran is used as animal feed in China extensively (En *et al.,* 2008).

There is a wide range of oxygen-free radicals and other reactive oxygen species (ROS), which include free radicals such as superoxide anion radicals (O_{2}^{-}), hydroxyl radicals (HO•), and non-free-radical species, such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$), which may form in the human body and in the food system. These radicals induce not only lipid peroxidation that causes deterioration of foods, but also cause oxidative damage by oxidizing biomolecules leading to cell death and tissue damage, such as atherosclerosis, cancer, emphysema, cirrhosis, and arthritis (Kehrer, 1993). Antioxidant agents can act against free radicals either by retarding their formation (preventive antioxidants) or by inactivating radicals in reaction medium (chain-breaking antioxidants) (Park *et al.,* 2001). Currently, the natural antioxidant α-tocopherol and some synthetic antioxidants such as butylated hydroxytoluene, butylated hydroxyanisole and propyl gallate are commonly used to act against free radicals in food

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and biological systems. However, the use of synthetic antioxidants in food products is under strict regulation owing to their potential health hazards (Hettiarachchy *et al.,* 1996).

Polyphenols are natural occurring substances and the most abundant antioxidants found in foods such as fruits, wine, tea, and cereals grains. Their total dietary intake could be as high as 1 g/day, which is much higher than that any polyphenols and known dietary antioxidants (Prashant *et al.,* 2005; Fardet *et al.,* 2008). Previous studies have shown that some phenolic compounds exhibit a wide range of pharmacological and medicinal properties, including anti-inflammatory, anti-carcinogenic, vasodilatory actions, which have been mostly attributed to their free radical scavenging, metal chelating, and antioxidant activities (Fardet *et al.,* 2008; Arpornsuwan *et al.,* 2010). They may play an important role in human health, reducing the risk of various degenerative diseases, such as cardiovascular diseases, osteoporosis, and cancer (Scalbert *et al.,* 2005).

Proximate analysis, nutritional, and functionality of foxtail millet were widely studied (Dykes and Rooney, 2006; Kamara *et al.,* 2009, 2010; Liang *et al.,* 2010). However, information on the phenolic antioxidant activity of foxtail millet bran is yet to be studied. Therefore, the objective of this research was to present the proximate composition, total phenolic content, and extraction yield of foxtail millet bran. The article focused also on the *in vitro* antioxidant assays, including reducing power of defatted foxtail millet bran extracts.

2. Material and Methods

2.1 Foxtail millet bran and reagents

Foxtail millet bran was purchased from a local producer (Hebei, China). 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), Folin-Ciocalteu's reagent and gallic acid were purchased from Sigma-Aldrich, Inc. (Shanghai, China). Wheat bran was donated by K. Zhu of the Cereal Research Group, School of Food Science and Technology, Jiangnan University, Wuxi China. All other reagents were of analytical grade.

2.2 Proximate analysis

The proximate analysis of foxtail millet bran and defatted foxtail millet bran was determined according to James (1995). The moisture content was determined by drying in an oven at 105°C until a constant weight was obtained. Ash was determined by weighing the incinerated residue obtained at 525°C after 4 hrs. Crude fat was extracted by the Soxhlet method with petroleum ether. The crude protein was determined by the micro-Kjeldahl method and using conversion factor of N × 6.25 and crude fiber was determined according to AOAC (1990). The carbohydrate content was estimated by subtracting the sum of percentage of moisture, crude fat, crude protein, fiber and ash contents from 100%.

2.3 Fatty acid analysis

Fatty acid of foxtail millet bran was determined according to the method of James (1995). Fat was extracted with methyl ether that was prepared directly with the treatment of the fat with sodium methoxide. Gas chromatography/mass spectrometry (GC/MS) system was used to identify and quantify the fatty acids of the product developed on a FINNIGAN TRACE MS gas chromatograph/mass spectra equipped with a 30 m x 0.25 mm Ov-1701 column. Column flow rate was 0.8 ml/min with helium as the carrier gas; split was 64 ml/min at the temperature of 270°C. The fatty acid methyl esters were identified by comparison with the retention times of NU CHECK Inc. standards (Elysian, 1L) and quantified by internal normalization.

2.4 Preparation of extracts

Five grams of defatted foxtail millet bran (DFMB) were extracted with 50 ml of water, ethanol and ethanol/water mixtures in different proportions. The solvent mixtures were used: ethanol/water (3:7 v/v), ethanol/water (1:1 v/v), and ethanol/water (7:3 v/v). The extraction was carried out at room temperature for 3 hrs under gentle stirring (Pérez-Jiménez *et al.,* 2008). The supernatant and the sediment were separated by centrifuging at 8,000 rpm at 4°C for 20 min using a centrifuge 5804R, Oppendorf AG 22331 (Hamburg, Germany). The residue was re-extracted with 25 ml of extraction solvents for 60 min and centrifuged under the same conditions. The combined supernatant was concentrated using a rotary evaporator at $\leq$ 50 °C. The extract was freeze-dried and stored in a brown desiccator until use. The dried extracts were weighed and the yield was calculated based on the weight of DFMB. The wheat bran extract was also used for comparison and it was extracted according to method described by Yu *et al.* (2003).

2.5 Determination of total phenolic content

The Folin–Ciocalteu method was used for total phenolic content determination as described by Vázquez *et al.* (2008). The Folin–Ciocalteu reagent (2.5 ml) was diluted with water (1:10, v/v), and mixed with 2 ml of 75 g/L aqueous solution of sodium carbonate. The resultant solution was added to 0.5 ml of an aqueous solution of millet bran extract. The mixture was kept for 5 min at 50°C before measuring the absorbance at 760 nm. The total phenolic content was determined from the calibration curve ($y=0.1536x–0.1433$; $R^2=0.9845$) of gallic acid standard solutions (1–20 mg/L) and expressed as mg gallic acid equivalent (GAE)/100 g of defatted foxtail millet bran extract.

2.6 Reducing power

The reducing power of DFMB extract was measured according to Wu *et al.* (2003). The sample was added to 2 ml

of 0.2 M phosphate buffer (pH 6.6) and 2 ml of 1% (w/v) potassium ferricyanide. The mixture was incubated at 50°C for 20 min, and then 2 ml of 10% (w/v) trichloroacetic acid (TCA) added. The mixture was centrifuged for 10 min at 3000 × *g*, and 2 ml of the supernatant was mixed with 2 ml of distilled water and 0.4 ml of 0.1% (w/v) $FeCl_3$. After reaction for 10 min, the absorbance of the solution was read at 700 nm. Increase in the absorbance of the reaction mixture indicated increased reducing power.

2.7 DPPH radicals scavenging activity assay

The scavenging effect of DFMB extract on DPPH free radical was measured according to the method of Shimada *et al.* (1992) with little modification. Two milliliters of each sample solution were added to 2 ml of 0.1 mM DPPH dissolved in 95% ethanol. The mixture was shaken and left for 30 min at room temperature, and the absorbance of resulting solution was read at 517 nm. A lower absorbance represents a higher DPPH scavenging activity. The scavenging effect was expressed as shown in the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{(Blank\ absorbance - Sample\ absorbance)}{Blank\ absorbance} \times 100 \quad (1)$$

2.8 Superoxide anion radicals scavenging activity assay

Superoxide anion radical (O_{2^-}) scavenging of DFMB extract was assessed using the method of Wang *et al.* (1994) with slight modification. Sample solution was mixed with 4.5 ml Tris-HCl (0.05M/L: pH 8.2). The reaction mixture was reacted with a solution containing 0.4 ml of 2.5 mM/L Pyrogalic acid, mixed and incubated at 25°C for 4 min. The reaction was stopped by adding two drops of 8 mM/L HCl. The absorbance of the reaction mixture was measured at 299 nm. The superoxide anion radical scavenging activity was calculated by the following equation:

$$\text{Superoxide anion scavenging activity (\%)} = \frac{(Blank\ absorbance - Sample\ absorbance)}{Blank\ absorbance} \times 100 \quad (2)$$

2.9 ABTS radicals scavenging activity assay

ABTS radical scavenging activity of DFMB extract was determined according to the method described by Re *et al.* (1999), with slight modification. A stock solution of ABTS radicals was prepared by mixing 5.0 ml of 7 mM ABTS solution with 88 µl of 140 mM potassium persulfate, and kept in the dark at room temperature for 12-16 hrs. An aliquot of stock solution was diluted with phosphate buffer (5 mM, pH 7.4) containing 0.15 M NaCl in order to prepare the working solution of ABTS radicals to an absorbance of 0.70±0.02 at 734 nm. A 65 µl aliquot of sample solution was mixed with 910 µl of ABTS radical working solution, incubated for 10 min at room temperature in the dark, and then absorbance was measured at 734 nm. The percent reduction of $ABTS^+$ to ABTS was calculated according to the following equation:

$$\text{ABTS (\%)} = \left(1 - \frac{absorbance\ of\ sample}{absorbance\ of\ control}\right) \times 100 \quad (3)$$

2.10 Statistical analysis

All experiments were conducted at least in triplicate. Analysis of variance (ANOVA) was performed and significant difference in mean values were evaluated by Tukey HSD multiple range test at ($p<0.05$) or ($p<0.01$) using SPSS version 18.0 (SPSS, Chicago, IL, USA).

3. Results and Discussions

3.1 Proximate composition

Consumption of whole grain products and dietary fiber has been shown to have health benefit, foxtail millet whole grain as well as foxtail millet bran contained significant amount of dietary fiber (Kamara *et al.,* 2009; Liang *et al.,* 2010). Table 1 shows the proximate chemical compositions of foxtail millet bran and defatted foxtail millet bran. Defatting affected significantly the protein content (14.82–12.93%). It was also observed that the ash content increased after the removal of fat (10.32–7.78%). The results from this work were in accordance with the data reported by Liang *et al.* (2010). Prior to defatting, foxtail millet bran contained 9.63% fat; however, a significant decrease was observed (1.85%) after defatting (Table 1). The high fiber content in foxtail millet bran (42.56%) is an indication that it could be a good source for edible fiber product. These results corroborated with data reported by Amissah *et al.* (2003) on rice bran variety.

3.2 Fatty acid

Storage temperature and moisture content are significant factors that affect fat acidity values. Fatty acid composition revealed that DFMB oil contained higher mono-unsaturated fatty acid than rice bran oil, peanut and olive oil (Table 1). The foxtail millet bran oil contained a large proportion of mono-unsaturated fatty acid with cholesterol lowering ability, but deficient in poly-unsaturated fatty acid, which contains essential fatty acids that are essential to humans (Liang *et al.,* 2010). The high content of mono-unsaturated fatty acid in foxtail millet bran oil (87% mono-unsaturated, 4% poly-unsaturated and 9% saturated fatty acid) is shown in Table 1. Hence, foxtail millet bran oil could be an immerging source of nutritional food compared to the known olive oil on the market, which also rich in mono-unsaturated fatty acid.

Table 1. Proximate composition of foxtail millet bran and comparison of foxtail millet bran oil.

Proximate composition (%)			Comparison of foxtail millet bran oil composition (%)			
	FMB[a]	Defatted FMB	Oil type	Monounsaturated fatty acid	Polyunsaturated fatty acid	Saturated fatty acid
Moisture[b]	8.47±0.16	7.51±0.20	FMB oil	87	4	9
Protein[b]	12.93±0.21	14.82±0.57	Rice bran[c]	47	33	20
Fat[b]	9.63±015	1.85±0.08	Olive[c]	77	9	14
Ash[b]	7.78±0.20	10.32±019	Peanut[c]	48	34	18
Fiber[b]	42.56±0.15	–	Soybean[c]	24	61	15
Carbohydrate[b]	18.63 ±0.87	65.50 ± 1.04	Grape seed[c]	14	77	9

[a] FMB: foxtail millet bran; [b] Values are means ± standard deviation of triplicate determinations;
[c] http://www.californiariceoil.com/nutrition.htm

3.3 Total phenolic content

Phenolics are one major class of phytonutrients that have been widely studied, thus they are well known antioxidants compounds that work in multiple ways to prevent disease (Ismail *et al.,* 2010). Total phenolic content of different DFMB extract varied significantly ($p<0.05$) among the extractions (Table 2), ranged from 29.39±1.36 to 21.49±3.26 GAE/100 g extract. The phenolic content was highest in 50% ethanol extract (29.39±1.36 GAE/100 g extract) and significantly ($p<0.05$) greater than the control (18.46±0.57 GAE/100 g extract), followed by aqueous extract (24.71±3.93 GAE/100 g extract), and the lowest was in 70% ethanol extract (21.49± 3.26 GAE/100 g). In the study of antioxidant activity in cereal grains, ethanol, and water were the most widely used solvents procedure. Ethanol was reported to be one of the best extraction solvent for antioxidant components (Yu *et al.,* 2003; Ismail *et al.,* 2010; Thitiorul *et al.,* 2010). In the current study the extraction yield were significantly greater ($p<0.05$) than the wheat bran samples. However, the highest extraction yield (35.79%) was found in 50% ethanol extract (Table 2). These results corroborate with work of Abdelrahman *et al.* (1984) and Vithal and Machewad (2006) on the millet extracts.

3.4 Reducing power

In this assay, the presence of antioxidants caused the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form, and the yellow color of the test solution changed to various shades of green and blue depending on the reducing power of each compound. The defatted foxtail millet bran extract of different ethanol concentration showed considerable amount of reducing activity. The reducing power of the DFMB extracts increased with the increasing concentration. Significant change was observed at the same sample concentrations (0.7 to 1 mg/ml) of the DFMB extracts (Figure 1). 1 mg/ml of the different percentage ethanol and aqueous extracts reached absorbance values of 0.37, 0.33, 0.43, and 0.31 corresponding to aqueous extract, 30%, 50%, and 70%, respectively. However, the positive control (BHT) showed higher (0.54) reducing ability although the sample did not show obviously high reducing capacity. Rao *et al.* (2010) reported higher reducing power of rice bran methanolic extracts then our results. This could be because of cereal type and extraction medium. The data indicate that foxtail millet bran extracts are capable of donating electrons, which can react with free radicals to convert them to a stable products and strongly inhibiting radical chain reaction.

3.5 IC_{50} values in antioxidant properties

Free radical scavenging is a preventive antioxidant activity and the scavenging activity can be used as the indication of prevention from increased oxidative stress, and hydroxyl radical scavenging activity. It was suggested that the millet bran extracts may be beneficial to the alleviation of

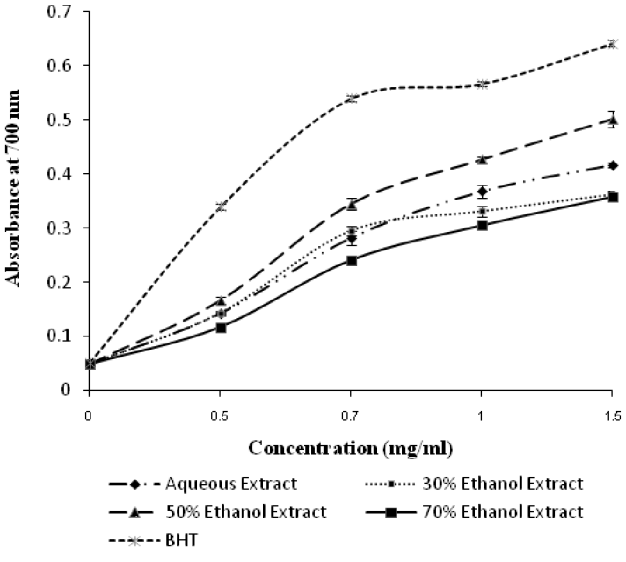

Figure 1. Reducing power of defatted foxtail millet bran extracts. Values are mean values ± standard deviation of three determinations.

oxidative stress, (Halliwell and Gutteridge, 1999; Fardet *et al.,* 2008). Several studies have revealed that the phenolic content in the plants are associated with their antioxidant activities, probably due to their redox properties, which allowed them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Ismail *et al.,* 2010).

The IC_{50} value (mg/ml) is the concentration of antioxidant at which 50% inhibition of free radical activity is observed (Scavenging Effect=Inhibitory Concentration or IC). The antioxidant properties on DFMB extracts were summarized in Table 2. The results were significantly different ($p<0.01$) and expressed as IC_{50} values for comparison. Effectiveness of antioxidant activities inversely correlated with IC_{50} values. With respect to the IC_{50} values when antioxidant activities were measured by DPPH and superoxide radical scavenging methods, the effectiveness of DFMB extracts were not in concordance in overall data, however, the ABTS activity of DFMB extracts were in ascending order: aqueous extract < 30% , < 50% and ~70% ethanol extract. Furthermore, the extracts were significantly different with the positive control (BHT) with regards to ABTS assay (Table 2). Previous investigations have demonstrated a correlation between antioxidant activity and total phenolic content, especially in the extracts of plant sources such as leaves, stem, aerial parts (Dykes and Rooney, 2006; Thitiorul *et al.,* 2010). It was understood that some other components besides phenolic such as proteins or peptides, polysaccharides, pigments, phospholipids in extracts were responsible for the antioxidant potential (Fardet *et al.,* 2008; Arpornsuwan *et al.,* 2010). IC_{50} value of DFMB aqueous extract (0.795±0.003 mg/ml ABTS assay) indicated the highest proton donating ability among the four other DFMB extracts (Table 2). Moreover, DFMB extracts in this study exhibited significant ($p<0.05$) correlation between antioxidant activity and phenolic contents. Indeed, the whole grains are also important dietary sources of water-soluble, fat-soluble, and insoluble antioxidants. Similar antioxidant activities have been reported in aqueous methanol or acetone extracts from oats, rice, wheat, and millet (Yu *et al.,* 2003; Serpen *et al.,* 2008; Rao *et al.,* 2010).

4. Conclusion

This study indicated that ethanol and water extracts of foxtail millet bran possessed potent antioxidant activities, including reducing power, scavenging abilities on DPPH, ABTS and superoxide radicals. Total phenolic and antioxidant activity of the extracts were both affected by the solvent proportions and defatting. DFMB increased significantly in proximate composition. Similarly the monounsaturated fatty acid of foxtail millet bran oil was higher than that of olive oil. Fifty percent ethanol was the most effective solvent for extraction of foxtail millet bran phenolic compounds. This could be related to the greater antioxidant stability in ethanol compared to aqueous ethanol systems. Further investigations on the relationship between phenolic content and antioxidant activity of foxtail millet bran extract are needed; in particular studies on isolation and structural elucidation of these antioxidative active constituents.

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Table 2. IC_{50} antioxidant activity, total phenolic content, and extraction yield of defatted foxtail millet bran (DFMB) extracts.

DFMB Extracts	IC_{50} of DPPH radical (mg/ml)*	IC_{50} of ABTS (mg/ml)*	IC_{50} of O_2^- (mg/ml)*	Phenolic content** (GAE/100g extract)	Extract Yield (% w/w)**
Aqueous	3.118 ± 0.008^{e}	0.795 ± 0.003^{a}	2.069 ± 0.003^{e}	24.71 ± 3.93^{ab}	30.19 ± 1.53^{c}
30% ethanol	0.652 ± 0.002^{b}	1.028 ± 0.003^{c}	0.158 ± 0.009^{a}	22.36 ± 1.35^{a}	33.49 ± 0.62^{cd}
50% ethanol	0.131 ± 0.002^{a}	1.765 ± 0.005^{d}	0.830 ± 0.009^{b}	29.39 ± 1.36^{b}	35.79 ± 1.10^{d}
70% ethanol	2.122 ± 0.003^{d}	1.881 ± 0.008^{e}	1.056 ± 0.010^{c}	21.49 ± 3.26^{a}	25.79 ± 1.10^{b}
BHT	1.670 ± 0.005^{c}	0.822 ± 0.013^{b}	—	—	—
Wheat bran	—	—	$1.109 \pm 0.025^{d-}$	18.46 ± 0.57^{a}	17.49 ± 0.50^{a}

IC_{50} value, the effective concentration at which the antioxidant activity was 50% and it was obtained by extrapolation from linear regression analysis. Data is expressed as mean ± standard deviation of three determinations. Mean with different superscript letters (a, b, c, d, e) within the column indicate significant difference *($p<0.01$), **($p<0.05$).

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