



Asian Journal of Chemistry; Vol. 28, No. 7 (2016), 1551-1556

ASIAN JOURNAL OF CHEMISTRY

<http://dx.doi.org/10.14233/ajchem.2016.19749>



Effect of Dehulling on Antioxidant Activity and Total Phenolic Content of Buckwheat (*Fagopyrum esculentum*) Flour

N. JINDAL* and D.C. SAXENA

Department of Food Engineering & Technology, Sant Longowal Institute of Engineering and Technology, Longowal-148 106, India

*Corresponding author: E-mail: navdeepjindal68@yahoo.com

Received: 30 November 2015;

Accepted: 17 December 2015;

Published online: 31 March 2016;

AJC-17847

Total phenolic content, total flavonoid content and antioxidant activity of free and bound extracts from whole and groats buckwheat flour were evaluated. The whole buckwheat flour was found to be better with regard to total phenolic content (12.60 mg GAE/g) and total flavonoid content (24.7 mg RUE/g). Among the two types of buckwheat flour, the antioxidant activity of the buckwheat flour as determined by DPPH (94.24 %), Lipid peroxidation (71.98 %), metal chelating activity (9.52 mg GAE/g) and found to be better than groat buckwheat flour. Antioxidant activities from both free and bound forms as determined by different extracting solvents were also found to be strongly correlated with the quantity of total phenolic content ($R^2 = 0.949$ in buckwheat flour, $R^2 = 0.934$ in groat buckwheat flour) and total flavonoid content ($R^2 = 0.997$ and 0.994 for buckwheat flour and groat buckwheat flour) respectively. The results of the given research revealed that VL-7 variety of buckwheat grown in Himalayan region of India possessed excellent phenolic and flavonoid content associated with antioxidant activities. This justified that buckwheat could be a good source to extract nutraceuticals and to formulate nutritious food products across the globe.

Keywords: Buckwheat flour, Antioxidant activity, Total phenols, Physio-chemical analysis.

INTRODUCTION

Buckwheat (*Fagopyrum esculentum*) is a major pseudo-cereal that is available throughout the year and can be used as an alternative to rice and wheat. The main functional property associated with buckwheat is the lack of gluten, which makes it a suitable substitute for those people suffering from gluten intolerance (celiac disease) due consumption of wheat or others gluten containing crops. Buckwheat is a nutritious and energizing crop that lowers the risk of developing higher systolic pressure. Buckwheat has been reported to prevent cardiovascular disease, obesity and 'Type 2' diabetes by reducing total serum cholesterol, low-density lipoprotein cholesterol (LDL) and lowers the formation of total cholesterol from HDL (health-promoting cholesterol) [1]. Buckwheat is a rich source of flavonoids, especially rutin. These flavonoids act as antioxidants that protect the human body against several chronic diseases. The rutin and other flavonoid compounds present in buckwheat play an important role in lowering excess accumulation of lipids, regulate proper blood flow, prevents blood platelets from excessive clotting and prevent oxidation of LDL into harmful cholesterol oxides [2]. Buckwheat is also rich in minerals, particularly magnesium, selenium and manganese which dilates blood vessels, thereby ensures

improvement in blood flow and nutrient delivery to different body parts [3]. Buckwheat is high in fiber, Vitamin E, phenolic acids, phytic acid and vitamin B. It also contains lignans that are plant chemicals associated with estrogen activity such as enterolactone; these lignans are linked with property of blocking the development of breast cancer [4]. Buckwheat contains all the eight essential amino acids, thus consumption of this cereal makes it one of the essential sources of vegetarian foods having complete protein [5]. Buckwheat serves as an alternative staple food and is rich source of natural antioxidants, which can be used for disease prevention and in formulation of nutritional foods and nutraceuticals. But also contain anti-nutritional factor like phytic acid, Tannin and trypsin inhibitor [6] and some toxic photochemicals like fagopyrins which are mainly distributed in the hull portion. Most of these factors are removed by dehulling. So buckwheat flour is either obtained by milling the dehulled grains (groats) or whole grains are which is then sieved to remove the bran. The flour is extensively used in various bakery and snack products as replacement flour. So, the study was conducted to determine the effect of dehulling on the phenolic and flavonoid content of whole (buckwheat flour) and groat buckwheat flour (BWGF) and to investigate the antioxidant properties of the buckwheat grown in Himalayan regions of India.

EXPERIMENTAL

Preparation of flour: Buckwheat grains (*Fagopyrum esculentum*) cultivar VL-7 were obtained from National Bureau of Plant Genetic Resources, Shimla, India. The grains were cleaned for shriveled, immature, damaged grains and impurities. The whole buckwheat flour of approximately 69 % extraction rate was prepared by milling the cleaned buckwheat in the hammer mill and sieving through 60 mesh sieve. Larger chunks of hulls and broken were removed. To obtain the buckwheat groat flour the whole buckwheat was dehulled using emery coated disk polisher and the whole and broken groats were then milled using hammer mill. Both the flours were kept under refrigerator at 5 ± 2 °C for further analysis.

Proximate analysis of buckwheat flour: The protein, fat, crude fiber and ash content of flours were determined with the standard methods [7]. Moisture was determined by means of infrared moisture meter. The carbohydrate content was calculated by subtraction of the remaining amount of these components from 100.

Starch estimation: The starch content was determined by Lane and Eynon method [8] with some modification. 5 g of buckwheat flour was mixed with 30 mL of water in a beaker. It was subjected to heat treatment at 60 °C for 25 min, followed by addition of 100 mL of 95 % ethanol with vigorous mixing. After filtering through Whatman filter paper no. 1, the residue was soaked in the 50 % ethanol solution for 1 h. The residue was collected in a round bottom flask with 100 mL of water and 20 mL of 6 M HCl. The flask after attaching with the condenser was heated for 2.5 h. The residue after cooling was neutralized by adding NaOH solution (40 %). Then 10 mL of Fehling solution taken into a conical flask was titrated against neutralized sample solution. After the observance of blue color, 3 drops of methylene blue indicator was added. The titration was continued till the observance of brick-red colour as end point.

The starch content was calculated by the following formula:

$$\text{Reducing sugar (\%)} = \frac{\text{Factor for Fehling solution} \times \text{Dilution}}{\text{Titre value} \times \text{Weight of sample}} \times 100$$

$$\text{Starch (\%)} = \text{Reducing sugar} \times 0.9$$

Amylose content of the buckwheat was determined from its starch by following the method of Morrison and Laignelet [9].

Extraction of free and bound buckwheat extracts: The lipid content of the buckwheat flour (whole and groat) was removed by extracting 0.5 g of flour sample with 10 mL of hexane twice. The lipid free sample was then extracted three times with 100 % pure methanol by shaking at room temperature in a water bath for 2 h. The methanol extract was then evaporated in a vacuum evaporator and the dried residues were diluted with methanol for determination of polyphenols and antioxidant capacity. The methanol extractable residues were then extracted by acetone diluted with 20 % water in the same way as given above. The remaining residues were then digested by 10 mL of 2 N NaOH for 2 h at room temperature. The pH of the digested mixture was then adjusted to 1 by

adding HCl. After the extraction of mixture by ethyl acetate, the mixture was evaporated under reduced pressure and the remaining residues were reconstituted by methanol and revealed for antioxidant properties

Determination of total phenolic content (TPC): The total phenolic content in both free and bound fractions of buckwheat was analyzed by using Folin-Ciocalteu assay as mentioned by Min *et al.* [10]. The prepared extract (0.05 mL) was mixed with 0.55 mL distilled water and 0.25 mL of 20 % Folin-Ciocalteu reagent. 0.5 mL of 0.5 M ethanolamine was added to the mixture after a period of 5 min and the resulting mixture was kept for 90 min at room temperature. The absorbance of the mixture was measured at 760 nm against the reagent blank and expressed in units of mg GAE/g.

Determination of total flavonoid content: Total flavonoid concentration (TFC) in both the free and bound fractions was determined as per the procedure of Min *et al.* [10] with slight modification. The extract solution (0.25 mL) was mixed with 1 mL of distilled water and 0.075 mL of 5 % sodium nitrite (w/v). After 5 min, 0.15 mL of 10 % AlCl₃ (w/v) was added to the mixture. The mixture was diluted with 0.5 mL 1 M NaOH after a period of 6 min, with subsequent addition of 0.5 mL distilled water. The resulting mixture was centrifuged at 5000 g for 10 min at room temperature and the absorbance was determined at 510 nm against the reagent blank. The results of total flavonoid content were expressed as mg RUE/g.

DPPH radical scavenging activity: Free radical scavenging activities of buckwheat extract (free and bound) was determined with the aid of DPPH radical as described by Sasidharan *et al.* [11] with slight modifications. To 0.1 mL of the extract solution, 3.9 mL of DPPH solution was added and mixed followed by an incubation period of 30 min in dark at room temperature. The absorbance of the resultant mixture was recorded at 515 nm and scavenging effect of DPPH radical was calculated by the following equation:

$$\text{DPPH radical scavenging (\%)} = \left(1 - \frac{A_{515\text{nm}} \text{ sample}}{A_{515\text{nm}} \text{ control}} \right) \times 100$$

Inhibition of lipid per oxidation in egg yolk homogenate: Inhibitions of lipid per oxidation in the egg yolk was determined using a modified method thiobarbituric acid-reactive species (TBARS) assay protocol in Badmus *et al.* [12]. 0.5 mL of egg yolk homogenate (10 % in distilled water, v/v) was mixed thoroughly with 0.1 mL buckwheat extract in a test tube with volume made up to 1 mL by distilled water. Then, 0.05 mL FeSO₄ (0.07 M) was added to the above mixture and incubated for 30 min, to induce lipid peroxidation followed by addition of 1.5 mL of 20 % acetic acid and 1.5 mL of 0.8 % TBA (w/v) in 1.1 % sodium dodecyl sulfate (SDS). Finally 0.05 mL of 20 % TCA was added and the mixture was thoroughly mixed followed by suspending in boiling water for heating up to 60 min. then, after cooling of tubes, 5 mL of butanol was added to each tube and centrifuged at 4000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm and the percent inhibition was calculated as:

$$\text{Inhibition (\%)} = \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

Determination of iron chelating capacity (ICC): The iron chelating capacity assay was used to determine the iron chelating capacity of the buckwheat extracts following the procedure of Min *et al.* [10]. The extract of free and bound form (0.05 mL) was mixed with 0.05 mL of 1 mM ammonium ferrous sulphate solution and 1.3 mL of 10 % ammonium acetate buffer (w/v). After 5 min, 0.05 mL of 6.1 mM ferrozine colour reagent was added and the absorbance was determined at 562 nm in a spectrophotometer after 10 min. The iron chelating capacity was calculated using a calibration curve of gallic acid solutions at different concentrations and expressed as mg gallic acid equivalents (GAE) per g.

Total antioxidant capacity by phosphomolybdenum reduction assay: Phosphomolybdate assay system was used to determine the total antioxidant activity of the buckwheat extracts to 0.3 mL of the extract solution, 3 mL of reagent solution prepared by using sulphuric acid (0.6 M), 28 mM of sodium phosphate and 4 mM ammonium molybdate was added. The mixture was then kept in a water bath maintained at 95 °C for a period of 90 min. After cooling to room temperature; absorbance of the mixture was done by spectrophotometer at 695 nm against reagent blank. Total antioxidant capacity was expressed as mMAA/g.

Determination of proanthocyanidins: Proanthocyanidins were quantified by the butanol/HCl depolymerization of the buckwheat extracts (methanol and acetone + water extracts and bound extracts) as determined by Porter *et al.* [13]. In a screw capped glass tube, 6 mL of butanol/concentrate HCl (95:5, v/v) and 0.1 mL of 2 % (w/v) solution of $\text{NH}_4\text{Fe}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ in 2 M HCl were added to 1 mL of individual extracts. The tube was vortex mixed and heated in water bath at 95 °C for 50 min. Absorbance of the reaction mixture was measured at 540 nm by U V. spectrophotometer. The quantification was done based on the calibration curve of catchin and expressed in units of mg catchin Equ./g.

Statistical analysis: All the results were carried out in triplicate and expressed as mean and standard deviation. Data were statistically analyzed by analysis of variance (ANOVA) and Duncan's Multiple Range test ($p < 0.05$) was done to determine significant differences among the results.

RESULTS AND DISCUSSION

Proximate compositions: The proximate and physico-chemical compositions of whole buckwheat flour and buckwheat groat flour are presented in Table-1. The moisture contents of the whole buckwheat flour was found to be 11.36 %, while in buckwheat groat flour it was observed to be 11.02 %. Protein contents for buckwheat flour and groat buckwheat flour were found to be 12.76 to 13.61 %. It has been cited from literatures, that the buckwheat flour contains a great amount of residual protein than wheat flour. Buckwheat flour is rich in albumin and globulin and contains lesser amount of prolamine. The glutelin content in buckwheat has also been reported to be much lower than that of wheat flour [14]. Dehulled buckwheat fractions have been reported to possess higher concentration of proteins [15]. The lipid content of groat buckwheat flour (2.38 %) was higher than buckwheat flour (2.03). It has been reported by Soral-Smietana *et al.* [16], that

TABLE-1
PROXIMATE COMPOSITION OF BUCKWHEAT FLOUR

Parameter (%)	Whole buckwheat flour	Groat buckwheat flour
Moisture content	11.36 ± 0.25 ^a	11.02 ± 0.22 ^a
Ash	1.86 ± 0.15 ^a	1.05 ± 0.20 ^b
Carbohydrate	67.14 ± 0.60 ^b	69.93 ± 0.63 ^a
Fat	2.03 ± 0.20 ^a	2.38 ± 0.20 ^a
Protein	12.76 ± 0.30 ^b	13.61 ± 0.15 ^a
Starch	51.52 ± 0.10 ^b	53.44 ± 0.10 ^a
Amylose	28.22 ± 0.33 ^b	29.39 ± 0.41 ^a
Amylopectin	71.38 ± 0.33 ^a	70.61 ± 0.14 ^b
Crude fibre	5.26 ± 0.32 ^a	2.43 ± 0.40 ^b

Results are expressed as mean value ± standard deviation of three determinations.
Means in rows with different superscript differ significantly ($p < 0.05$).

buckwheat flour contains higher content of free lipids than that of bound lipids. The higher content of ash in buckwheat flour could be due to the residual hull associated with it. Value of crude fibre in buckwheat flour (5.26 %) could be due to large amount of hull in comparison to groat buckwheat flour (2.43 %).

Starch is an energetic storage component in the endosperm of buckwheat grains varies from 59 to 70 % of the dry mass, depending upon variable climatic and cultivation conditions [17]. The current results of starch analysis of whole buckwheat flour and buckwheat groat flour were found to be 51.52 and 53.44 %. Amylose content of buckwheat starch were reported to fluctuate between 15 % and 52 % and its degree of polymerization ranged from 12 to 45 glucose units [18]. The higher amount of amylose content in buckwheat (29.39 %) in given studies justified their use in patients suffering from diabetic complications.

Total phenolic content (TPC): The total phenolic content (TPC) of whole buckwheat flour and buckwheat groat flour as determined by the Folin-Ciocalteu method was expressed in unit of mg GAE/g of dry weight and the results are presented in Table-2. Total phenolic content of the whole buckwheat flour in bound form and in extract of methanol varied from 0.88 to 10.33 mg GAE/g respectively. The total phenolic content of whole buckwheat flour was found to be 12.60 mg GAE/g, which included the sum of free phenolic content as extracted by pure methanol and acetone plus water extract (1.38 mg GAE/g) together with bound phenols as extracted by ethyl acetate after digestion of the remaining materials. The higher total phenolic content in buckwheat flour may be due to the presence of significant amount of phenolic compound like ferulic acid, chlorogenic acid, vanillic acid, *trans*-ferulic acid, *p*-anisic acid, salicylic acid, methoxycinnamic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid and diferulate, which are not found in significant amounts in fruits and vegetables [19,20].

Hulls of buckwheat had higher phenolic content [21], which reflects the higher total phenolic content in whole buckwheat flour. The total phenolic acids in buckwheat had been reported to account for 20 to 30 % of esterified phenolic acid [22]. For buckwheat groat flour, the total phenolic content was found to be 8.57 mg GAE/g that was significantly less than buckwheat flour. The free content of total phenolic content in buckwheat groat flour as determined by methanol and

TABLE-2
ANTIOXIDANT PROPERTIES OF BUCKWHEAT FLOURS

	Whole buckwheat flour	Buckwheat groat flour
Total phenolic content (mg GAE/g)		
Methanol	10.33 ± 0.57 ^a	6.86 ± 0.02 ^b
Acetone + water	1.38 ± 0.53 ^a	0.90 ± 0.02 ^a
Bound	0.88 ± 0.01 ^a	0.80 ± 0.02 ^b
Total	12.60 ± 1.11 ^a	8.57 ± 0.02 ^b
Total flavonoid content (mg RUE/g)		
Methanol	17.70 ± 0.01 ^a	15.15 ± 0.01 ^b
Acetone + Water	5.37 ± 0.02 ^a	3.58 ± 0.01 ^b
Bound	1.70 ± 0.02 ^a	0.59 ± 0.01 ^b
Total	24.70 ± 0.05 ^a	19.30 ± 0.04 ^b
Lipid peroxide inhibition (%)		
Methanol	56.58 ± 0.20 ^a	51.38 ± 0.01 ^b
Acetone + water	5.40 ± 0.01 ^a	4.73 ± 0.05 ^b
Bound	10.01 ± 0.15 ^b	10.66 ± 0.57 ^a
Total	71.98 ± 0.10 ^a	66.78 ± 0.61 ^b
DPPH radical scavenging activity (%)		
Methanol	67.38 ± 0.02 ^a	56.58 ± 0.01 ^b
Acetone + water	22.15 ± 0.03 ^b	26.65 ± 0.02 ^a
Bound	4.70 ± 0.01 ^b	4.77 ± 0.01 ^a
Total	94.24 ± 0.05 ^a	88.00 ± 0.02 ^b
Pro anthocyanidin content (mg catchin Equ/g)		
Methanol	0.73 ± 0.11 ^a	0.57 ± 0.01 ^a
Acetone + water	0.35 ± 0.01 ^b	0.43 ± 0.01 ^a
Bound	0.05 ± 0.01 ^a	0.07 ± 0.01 ^a
Total	1.14 ± 0.12 ^a	1.08 ± 0.01 ^a

Results are shown as mean ± S.D. Means in a same row with same superscript do not differ significantly (p < 0.05)

acetone + H₂O was shown to be 6.86 to 0.90 mg GAE/g. A strong positive correlation between total phenolic content and DPPH radical scavenging was observed ($R^2 = 0.989$ in whole buckwheat flour and 0.949 in buckwheat groat flour). The difference in phenolic content between whole buckwheat flour and buckwheat groat flour could be attributed to the removal of hull. Similar findings were reported for other cereal grains due to varying degree of polishing depending on their consumer choice [23].

Total flavonoid content (TFC): Total flavonoid content was found to be higher in whole buckwheat flour (24.7 mg RUE/g) than buckwheat groat flour (19.3 mg RUE/g) as given in Table-2. Flavonoids in buckwheat have received a greater attention due to their flavonoid contents, particularly the rutin. The six flavonoids identified in buckwheat are rutin, orientin, vitexin, quercetin, isovitexin and isoorientin, in which rutin attributed most of the flavonoid content [24]. Flavonoid content in buckwheat varies according to their species and the growing environment. A value of total flavonoid content in the given variety was found to be higher than *Fagopyrum esculentum* (10 mg/g) but lower than that of *Fagopyrum tataricum* (40 mg/g) as reported by Li and Zhang [25]. The higher content of polyphenols in buck wheat are strongly interacted with proteins that account for the lower solubility of these proteins [26]. Flavonoids in buckwheat are highly present in the hulls which are over 3 times the amount present in seed [27]. This validated the results of buckwheat flour in the given work. The correlation between total flavonoid content and DPPH was found to be positive ($R^2 = 0.997$ and 0.99). The bound

total flavonoid content in buckwheat flour was found to be higher (1.70 mg RUE/g) as compared to groat buckwheat flour (0.59 mg RUE/g). The flavonoids in buckwheat have been reported to ensure higher antioxidant activity, which validated their uses in pharmaceutical and nutraceuticals [27].

DPPH radical scavenging activity (%): Antioxidative activity, as determined by the DPPH radical scavenging activity showed a significant difference between buckwheat flour and groat buckwheat flour. The free and bound scavenging activities in buckwheat flour were found to be 76.59 % (in methanol), 5.40 % in acetone plus water and it showed the value of 4.70 % in bound form giving a total value of 94.24 %. While the DPPH scavenging activity showed a total value of 88.0 % in groat buckwheat flour, which included the free DPPH activity in methanol (56.58 %) and acetone + H₂O (26.65 %) coupled with DPPH activity in bound form (4.77). DPPH activities in buckwheat were reported to be higher than in other cereal grains (wheat) due to their higher polyphenolic content [28]. Holasova and co-workers [29] also reported the phenolic compounds in buckwheat had been reported to possess higher anti-oxidative activity than the phenolic components present in oats and barley. The significantly higher total phenolic content in buckwheat flour reflects the higher DPPH scavenging activity in buckwheat flour. The main flavonoid compound rutin, present in buckwheat have been reported by researchers to exhibit a strong DPPH radical scavenging activity [30].

Lipid peroxide inhibition (%): Inhibitory capacity of the buckwheat extracts as determined by using egg yolk homogenate revealed a significant difference. Wherein whole buckwheat flour (71.98 %) had a higher value than buckwheat groat flour (66.78 %). These results correlate well with the values of total phenolic content and total flavonoid content. The contrasting point revealed here is that the inhibition capacity of bound material in whole buckwheat flour (10.01 %) is lower than that observed in buckwheat groat flour (10.66 %). The buckwheat extracts react with the peroxy radicals, which propagate of the auto-oxidation of fat resulting in termination of the chain reaction [31]. The results of lipid peroxide inhibition in buckwheat indicate that antioxidant compounds in buckwheat act as strong inhibitors. The higher antioxidant capacity of buckwheat could be linked with their high polyphenols content, especially with their higher content of rutin [32]. Inhibition of lipid peroxidation also depicted positive correlations with total phenolic content ($R^2 = 0.993$ and 0.982) and total flavonoid content ($R^2 = 0.937$, 0.905), respectively. Phenolic compounds also determine their antioxidant activity by directly scavenging some radicals like hydroxyl, peroxy and superoxide radicals, which can terminate the chain reaction in lipid oxidation resulting in inhibition of lipid peroxidation [33].

Iron chelating activity (mM GAE/g): As shown in Fig. 1, the total iron chelating activity is significantly higher in whole buckwheat flour (9.52) and lower in buckwheat groat flour (8.57). Where in the iron chelating activity in methanol and acetone (with water) extracts of whole buckwheat flour are 3.87 and 4.23, while the values of buckwheat groat flour in the extracts were 3.26 (methanol) and 4.52 (acetone + water). The chelating activity of bound extracts of whole buckwheat

flour (1.32) and buckwheat groat flour (0.97) were observed to be lower than that of DPPH activity and lipid peroxidation inhibition. Some phenolic compounds have been reported to bind with pro-oxidant iron, thereby suppressing the formation of pro-oxidant iron free radical while simultaneously maintaining their capacity to scavenge these iron free radicals [34]. Iron chelating is considered as an important mechanism of determining antioxidant property based on retardation of iron-catalyzed oxidation. Ferrous ions, which are the most reactive pro-oxidants stimulate lipid peroxidation by means of Fenton reaction [35]. It also accelerates lipid peroxidation by decomposing lipid hydroperoxides into peroxy and alkoxy radicals, which abstracts hydrogen resulting into perpetuation of the chain reaction in lipid peroxidation process [36]. The higher concentration of flavonoids and proanthocyanidins in buckwheat flour could account for their higher iron chelating capacity, as flavonoids and proanthocyanidin have been considered as strong iron chelating agent [37].

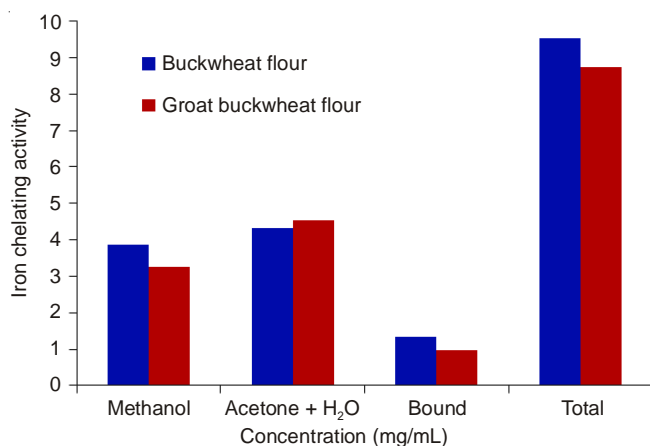


Fig. 1. Iron chelating activity of buckwheat flour and groat buckwheat flour

Phosphomolybdenum assay (mM AA/mL): The antioxidant activity of buckwheat as determined by phosphomolybdenum assay is based on the reduction of Mo(VI) to Mo(V) by the antioxidants present in extracts of buckwheat with the formation of a green phosphate, Mo(V) complex having maximum absorption at 695 nm according to The Beer-Lambert law [38]. The phosphomolybdenum method is simple and rapid in procedure; it was used to determine its application to the buckwheat extracts. A significant difference ($P > 0.05$) was observed between the whole buckwheat flour and buckwheat groat flour results. Here the antioxidant activity in buckwheat groat flour (39.97 mM AA/mL) was found to be higher than whole buckwheat flour (34.09 mM AA/mL). This contrasting results in antioxidant activity can be due to the use of different solvents for extraction of buckwheat, which had been found to effect the composition of the extracting compounds and hence the antioxidant activities of the buckwheat flour and groat buckwheat flour extracts [39].

Proanthocyanidin content (mg catchin Equ/g): The proanthocyanidins content varied from 1.14 to 1.04 in whole buckwheat flour and buckwheat groat flour respectively. The proanthocyanidin content in free form as estimated by methanol was higher in whole buckwheat flour (0.73), while acetone + water extracts showed higher proanthocyanidin

content in buckwheat groat flour (0.43). The proanthocyanidin content in bound form was higher in buckwheat groat flour (0.07) and lower in whole buckwheat flour (0.05). The difference in proanthocyanidin content was reflected in the total polyphenol content of buckwheat. Proanthocyanidins have been reported to increase antioxidant property in cereals [40]. Proanthocyanidins represent one of the classes of flavonoids having different solubility that exerts their action in the gut [41]. The polymeric proanthocyanidins may be degraded into smaller phenolic components by the colonic microflora residing in the human body [42] and prevent the human body from occurrence of plasma postprandial oxidative stress [43].

Conclusion

The whole buckwheat flour (BWF) was found to be better with respect to total phenolic content and total flavonoid content. Total phenolic content, total flavonoid content and antioxidant activity of free and bound extracts from whole and groat buckwheat flour were found to be significant. The results of the present study revealed that VL-7 variety of buckwheat grown in Himalayan region of India possessed high phenolic and flavonoid content associated with antioxidant activities. This suggests that buckwheat could be used a raw material to extract nutraceuticals and to formulate functional foods across the globe.

ACKNOWLEDGEMENTS

The authors acknowledge Dr. J.C. Rana from National Bureau of Plant Genetic Resources, Shimla, India for providing the buckwheat grains.

REFERENCES

1. D.R.M. Van, F.B. Hu, L. Rosenberg, S. Krishnan and J.R. Palmer, *Diabetes Care*, **29**, 2238 (2006).
2. S. Kreft, M. Knapp and I. Kreft, *J. Agric. Food Chem.*, **47**, 4649 (1999).
3. L. Bonafaccia, N. Gambelli, N. Fabjan and I. Kreft, *Food Chem.*, **83**, 1 (2003).
4. J.E. Cade, V.J. Burley and D.C. Greenwood, *Int. J. Epidemiol.*, **36**, 431 (2007).
5. D. Gabrovska, V. Fiedlerova and M. Holasova, *Food Nutr. Bull.*, **23 Suppl.**, 246 (2002).
6. J. Lott, J.S. Greenwood and G.D. Batten, in eds.: J. Kigel and G. Galili, Marcel Dekker, New York, pp. 215-235 (1995).
7. AOAC, Official Methods of Analysis, Association of Official Analytical Chemists, Washington, DC, edn 15 (2000).
8. J.H. Lane and L. Eynon, *J. Soc. Chem. Ind. Trans.*, **42**, 32 (1923).
9. W.R. Morrison and B. Laignelet, *J. Cereal Sci.*, **1**, 9 (1983).
10. B. Min, A.M. McClung and M.H. Chen, *J. Food Sci.*, **76**, C117 (2011).
11. S. Sasidharan, Y. Chen, D. Saravanan, K.M. Sundram and L.Y. Latha, *Afr. J. Tradit. Complement. Altern. Med.*, **8**, 1 (2011).
12. J.A. Badmus, T.O. Adedosu, J.O. Fatoki, V.A. Adegbite, O.A. Adaramoye and O.A. Odunola, *Acta Polon. Pharm.*, **68**, 23 (2011).
13. L.J. Porter, L.N. Hrstich and B.G. Chan, *Phytochemistry*, **25**, 223 (1985).
14. W. Yimin, Z. Guoquan and L. Zhixi, Proc. 5th Int. Symp. on Buckwheat, Taiyuan, China, pp. 502-510 (1992).
15. B. Krkoskova and Z. Mrazova, *Food Rev. Int.*, **38**, 561 (2005).
16. M. Soral-Smietana, L. Fornal and J. Fornal, *Food/Nahrung*, **28**, 483 (1984).
17. J. Qian and M. Kuhn, *Starch/Stärke*, **51**, 81 (1999).
18. C.G. Campbell, Buckwheat: *Fagopyrum esculentum* Moench, IPGRI Rome, pp. 1-93 (1997).
19. S. Biswas, D. Sircar, A. Mitra and B. De, *Nutr. Food Sci.*, **41**, 123 (2011).
20. B.C. Xu, G. Xiao and X.L. Ding, *Food Ferment. Ind.*, **12**, 32 (2002) (in Chinese).

21. Y.S. Velioglu, G. Mazza, L. Gao and B.D. Oomah, *J. Agric. Food Chem.*, **46**, 4113 (1998).
22. B.D. Oomah, C.G. Campbell and G. Mazza, *Euphytica*, **90**, 73 (1996).
23. S. Butsat and S. Siriamornpun, *Food Chem.*, **119**, 606 (2010).
24. D. Dierych-Szostak and W. Oleszek, *J. Agric. Food Chem.*, **47**, 4384 (1999).
25. S.Q. Li and Q.H. Zhang, *Crit. Rev. Food Sci. Nutr.*, **41**, 451 (2001).
26. B.T. Metzger, D.M. Barnes and J.D. Reed, *J. Agric. Food Chem.*, **55**, 6032 (2007).
27. B.D. Oomah and G. Mazza, *J. Agric. Food Chem.*, **44**, 1746 (1996).
28. I.J. Sedej, M.B. Sakac, A.C. Misan and A.I. Mandic, *Matica Srpska Proc. Nat. Sci.*, **118**, 59 (2010).
29. M. Holasova, V. Fiedlerova, H. Smrcinova, M. Orsak, J. Lachman and S. Vavreinova, *Food Rev. Int.*, **35**, 207 (2002).
30. M. Watanabe, Y. Ohshita and T. Tsushida, *J. Agric. Food Chem.*, **45**, 1039 (1997).
31. F. Shahidi, P.K. Janitha and P.D. Wanasundara, *Crit. Rev. Food Sci. Nutr.*, **32**, 67 (1992).
32. I. Kreft, N. Fabjan and K. Yasumoto, *Food Chem.*, **98**, 508 (2006).
33. K. Marimuthu, P. Magesh, S.G. Jasbin, R. Dhanalakshmi, K. Ponraj, K. Lavanya and R. Kalimuthu, Antioxidant Potential of Brown Rice, pp. 134-137 (2014).
34. J.F. Moran, R.V. Klucas, R.J. Grayer, J. Abian and M. Becana, *Free Radic. Biol. Med.*, **22**, 861 (1997).
35. X.Q. Zha, J.H. Wang, X.F. Yang, H. Liang, L.L. Zhao, S.H. Bao, J.P. Luo, Y.Y. Xu and B.B. Zhou, *Carbohydr. Polym.*, **78**, 570 (2009).
36. B. Halliwell, *Am. J. Med.*, **91**, 14 (1991).
37. L. Mira, M.T. Fernandez, M. Santos, R. Rocha, M.H. Florencio and K.R. Jennings, *Free Radic. Res.*, **36**, 1199 (2002).
38. Y. Pan, K. Wang, S. Huang, H. Wang, X. Mu, C. He, X. Ji, J. Zhang and F. Huang, *Food Chem.*, **106**, 1264 (2008).
39. K.X. Zhu, C.X. Lian, X.N. Guo, W. Peng and H.M. Zhou, *Food Chem.*, **126**, 1122 (2011).
40. A.M. Reddy, V.S. Reddy, B.E. Scheffler, U. Wienand and A.R. Reddy, *Metab. Eng.*, **9**, 95 (2007).
41. A. Fardet, E. Rock and C. Remesy, *J. Cereal Sci.*, **48**, 258 (2008).
42. A.-M. Aura, I. Mattila, T. Seppänen-Laakso, J. Miettinen, K.-M. Oksman-Caldentey and M. Oresic, *Phytochem. Lett.*, **1**, 18 (2008).
43. F. Natella, F. Belevi, V. Gentili, F. Ursini and C. Scaccini, *J. Agric. Food Chem.*, **50**, 7720 (2002).