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A Comparative Study on Nutritional Profile and Antinutrients of Buckwheat Fractions (*Fagopyrum esculentum*)

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A B S T R A C T

Buckwheat (*Fagopyrum esculentum*) is an annual crop, it is a pseudo cereal but its grains belong to cereals because of their similar use and chemical composition. Buckwheat grains and other tissues contain numerous neutraceutical compounds. A Comparative study on Nutritional profile and Antinutrients of buckwheat fractions was conducted at Department of Food & Nutrition, College of Home Science, Maharana Pratap University of Agriculture & Technology Udaipur, Rajasthan, India. The chemical analysis of buckwheat fractions buckwheat whole (BW), buckwheat groats (BG) and buckwheat husk (BH)for proximate composition revealed significant difference for moisture, fat, ash, protein, fibre and energy. Protein was significantly higher in BG (14.88g/100g) than BW (11.34g/100g) and BH (9.91g/100g). It was observed that all three fractions of buckwheat exhibited almost similar values of carbohydrate content which ranged from 66.35g/100g in BW to 71.25g/100g in BH. The significant difference was found between fractions for calcium, Iron, and Zinc. In case of calcium, BH recorded higher value 149.66 ppm than BW and BG (76.80 ppm and 38.13 ppm). The anti-nutritional factors viz., tannin and phytic acid were analyzed in all flour fractions. Tannin content was found to be highest in BH (5.54%) than BW (4.15%) and BG (4.15%). The phytic acid content was found lowest in BG (6.23%) than BW (18.36%) and BH (18.30%) and the difference was significant ($p \geq 0.05$).

Introduction

Buckwheat is produced in many parts of the world and has long been an important part of the human diet. Buckwheat has a triangular seed, which is covered by a hull (pericarp). The exact shape, size, and colour of the seed may vary depending on the species and variety. The hull may be a glossy or dull brown, black or grey. The dehulled buckwheat seed, called the groat, resembles the cereal kernel in its gross chemical composition and structure. The first layer of the groat is aonecell thick testa layer (seed coat), which is light green in colour. Under the testa is a one-cell aleurone layer, which surrounds the starchy endosperm. The inner portion of groat consists of a spermaderm and an endosperm. Later, it was suggested by Krtov (1963) that buckwheat originated in temperate central Asia from where it has migrated to other countries of the region. The perennial wild species *Fagopyrum cymosum*, native to China and India was considered to

be the progenitor of the two commonly cultivated species, *Fagopyrum esculentum* (Common buckwheat) and *F. tataricum* (Tatary buckwheat). Hindi name for buckwheat (*Fagopyrum esculentum*) is "Kutu" and it's an ancient crop of India cultivated extensively in the Himalayan region extending from Jammu and Kashmir in the north-west to Arunachal Pradesh in the north eastern region. Buckwheat has gained an excellent reputation for its nutritious qualities in the human diet.Its renewed popularity stems from its many bioactive components, which have been shown top rovide various health benefits much sought after in natural foods. Buckwheat flour contains various kinds of vitamins, such as B1, B2, and niacin, at relatively high levels (Pomeranz, 1983). Buckwheat protein consists of 18.2% albumin, 43.3% globulin, 0.8% prolamin, 22.7% glutelin, and 5.0% other nitrogen residue (Javornikand Kreft, 1984).

Buckwheat contains many flavonoid compounds, known for their effectiveness in reducing the blood cholesterol, keeping capillaries and arteries strong and flexible, and assisting in prevention of high blood pressure (Santos *et al,* 1999). Buckwheat proteins, like dietary fibre, can suppress the development of colon cancer (Lipkin *et al.*, 1999). The content of TDF in groats may range from 5 to 11%. Bran fractions obtained by milling of buckwheat are especially enriched in dietary fibre (13-16%), but buckwheat flours contain considerably lower amounts of fibre (1.7-8.5%) (Steadman *et al.*, 2001).

Buckwheat flour can be avaluable ingredient in diets or food products for celiac patients. It is observed that Buckwheat is a nutritious food having therapeutic role in diseases like Diabetes, hypertension, cancer, constipation and celiac disease. It is a good source of protein, vitamins, and minerals bioactive components like flavonoids makes it a boon for health.

Materials and Methods

The present study was conducted at Department of Food & Nutrition, College of Home science, Maharana Pratap University of Agriculture & Technology Udaipur, (Rajasthan).Buckwheat sample as whole (BW) and Buckwheat groats (BG) purchased from local market of Udaipur (Rajasthan) in a single lot to avoid varietal difference. The samples are shown in plate 1.Sample was stored in airtight container Buckwheat whole (BW) cleaned separately by sieving for removal of dirt, stones and stored in airtight container. ZanduParad Tablets (covering with a piece of cotton cloth) added (2 tablets for 1 kg seed). Every 2-3 months interval samples were spread in sunlight and again stored.

Nutritional components: Buckwheat whole (BW), Buckwheat groats (BG) and buckwheat husk (BH) were analyzed for nutritional content along with buckwheat whole and buckwheat groats. Buckwheat husk was also analyzed as most trace elements are concentrated in bran (Bonafaccia *et al.*, 2003). Buckwheat husk (BH) was obtained by grinding buckwheat whole in a grinder for 2-3 minutes and husk removed manually by hand. About 50-60 percent part of buckwheat was separated as buckwheat husk (BH).

Nutritional evaluation of the buckwheat whole (BW) was done for their proximate composition and mineral estimation (calcium, iron, zinc, copper). Anti-nutritional factors (tannins and phytates) were also analyzed. Standard procedures were used for the estimations. Percentage carbohydrate and energy contents were determined by calculation using difference method respectively. The procedures have been described here under:

Proximate composition

It is the determination of a group of closely related compounds together. It includes determination of amount of moisture, protein, fat (ether extract), ash and fiber with nitrogen free extract and carbohydrates being estimated by subtracting the sum of these five percentages from 100.

Moisture

It is the major component of food. The moisture content of any food is determined not only to analyze the chemical composition of food material on moisture free basis but also to assess the shelf life of the products. Moisture content of samples was analyzed by the method described by NIN (1983).Ten gram sample was weighed in a dried and weighed petri dish. The weight of the sample along with the petri dish was taken at regular intervals until a constant weight was obtained. The moisture percentage was calculated using following formula:

$$\text{Moisture (g/100g)} = \frac{\text{Initial weight (g) - Final weight (g)}}{\text{Weight of the sample (g)}} X100$$

Crude protein

The protein nitrogen is converted into ammonium sulphate by boiling with concentrated sulphuric acid. It is subsequently decomposed by the addition of excess alkali and the liberated ammonia is absorbed into boric acid solution containing an indicator by steam distillation. Ammonia forms a loose compound, ammonium borate with boric acid, which is titrated directly against standard HCl. The protein content of food stuff is obtained by estimating the nitrogen content of the material and multiplying the nitrogen content by the factor 6.25 (NIN, 1983).

Kjel plus nitrogen estimation system was used to estimate the amount of nitrogen in the samples. 0.2 g moisture free sample was transferred to the digestion tube. Ten ml of concentrated sulphuric acid and 3 g catalyst mixture (5 parts of K2SO4 + 1 part of CuSO4) was added and was left overnight. The tubes were then placed in a pre-heated digestion block. The digestion block was pre heated to 60°C for 10 minutes. Once the digestion tubes were placed, temperature was further increased to 100°C and samples were kept until the colour of the samples turned bluish green or colorless. Digested samples were taken for distillation where the ammonium radicals were converted to ammonia under excess alkali post neutralization of acid in the digested samples with 40 per cent sodium hydroxide. Mixed indicator (methyl red + methyl blue) was added to the solution and titrated with the standardized N/10 HCl. The titration value was determined and the following formula was used to estimate the amount of nitrogen liberated:

$$\text{Nitrogen (g/100g)} = \frac{\text{14.01xNormality of HCL (0.1) x (TV-BV}}{\text{SW (gm)}} X100$$

Crude fat

Fat was estimated as crude ether extract of moisture free sample by the method given by Jain and Mogra (2006). Fat content of the sample was estimated on Soxhlet Plus system, which works on the principle of improved soxhlet method. Weighed amount of moisture free sample (5 g) was placed in a thimble. The thimble was inserted in the thimble holder to be kept in an already weighed beaker and 80 ml petroleum ether (60-80˚C) was poured in the beaker. The beakers were loaded in the system and temperature was set at100˚C. The process was left to operate for 120 minutes and the temperature was increased to the

recovery temperature, which was twice the initial boiling temperature. Rinsing was thus done twice in order to collect the remaining fat in the sample. Beakers were taken out and put Nitrogen (g/100g) = 14.01 x Normality of HCL (0.1) x (TV-BV)SW (gm)x 100in a hot air oven. Thimble holders were removed from the beakers and the beakers were weighed. The amount of fat present in the sample was calculated using the following formula:

$$\text{Fat (g/100g)} = \frac{\text{Weight of ether extract fat (B-A)}}{\text{Weight of sample (gm)}} \times 100$$

Ash

Ash was estimated by the method given by Jain and Mogra (2006). Five grams of moisture free sample was weighed in previously heated, cooled and weighed crucible. Sample was then completely charred on the hot plate, followed by heating in muffle furnace at 6000C for 5 hours. The crucible was cooled in desiccators and weighed. The process was repeated till constant weights were obtained and the ash was almost white or grayish in color. Ash content of samples was calculated using following formula:

$$\text{Ash (g/100g)} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample taken (g)}} \times 100$$

Crude fibre

Fibre is an insoluble vegetable matter indigestible by proteolytic and diastatic enzymes and cannot be utilized except by microbial fermentation. It is usually composed of cellulose, hemicelluloses and lignin. Crude fiber estimation was done as per the method given by 3 gram of moisture and fat free sample was placed in 500 ml beaker and boiled with 200 ml of 1.25 per cent sulphuric acid for thirty minutes. The volume was kept constant during boiling by adding hot distilled water. This was filtered through muslin cloth and the residue was washed with hot distilled water till free from acid. The residue was then transferred to same beaker and boiled for 30 minute with 200 ml of 1.25 per cent sodium hydroxide solution. After boiling, mixture was filtered through muslin cloth and the residue was washed again with hot distilled water till free from alkali followed by washing with 50 ml alcohol and ether. Then it was taken into a crucible (it was weighed before as W1) and residue was dried in an oven at 1300C for 2-3 hours, cooled and weighed (W2). Heat in muffle furnace at 6000C for 2-3 hours, then cool and weigh again (W3).

Carbohydrate

The carbohydrate content of the sample on dry weight basis was calculated by difference method (Jain and Mogra 2006) as given below:

Carbohydrate (g/100g) = 100 – (moisture + crude fibre + ash + protein + fat)

Energy

The energy value of sample was calculated using physiological fuel value i.e. 4, 9, 4 kcal per gram of protein, fat and carbohydrate respectively.

Energy (kcal/100g) = [(% protein x 4) + (% carbohydrate x 4) + (% fat x9)]

Mineral profile

Mineral solutions of selected samples were prepared by wet ashing method compiled by Jain and Mogra (2006). The plant material was digested with a mixture of acids to form a clear white precipitate which was then dissolved in water and made up to a definite volume. An aliquot from this was used for determination of selected minerals.

Wet ashing

One gram moisture free sample was taken in a digestion tube and 5 ml of concentrated HNO3 was added to it and was left overnight. It was then heated slowly for 30 minutes and cooled. Five ml of perchloric acid (70%) was added and heated over digestion block until the particles were completely digested and the solution became clear. After digestion, volume of digested matter was made up to 50 ml with double distilled water. Prepared mineral solution was stored in makeup bottles and mineral analysis was done by atomic absorption spectrophotometer (AAS4141)

Anti- nutritional factors

The nutritional quality and digestibility of plant nutrients is affected by the presence of anti nutritional factors. The presence of these anti-nutrients was analyzed in selected maize varieties.

Total tannin estimation

Total tannin content of the samples was estimated using the method of Atanassova and Christova (2009).Sample preparation- Three g of the sample was mixed with 250 ml distilled deionized water (dd H2O) and kept for 4 hours at room temperature and filtered in volumetric flask with filter paper. Tannin Essay-Twenty five ml infusion was measured into 1 litre conical flask then 25ml of indigo solution and 750 ml distilled deionized water was added 0.1 N aqueous solution of potassium permanganate was used for titration till the blue color of solution changes to green color. Further few more drops were added until solution becomes golden yellow. Standard solution of indigo carmine was prepared as follows- six gm indigo carmine was dissolved in500 ml of distilled deionized water by heating, after cooling 50 ml of 95-97% sulphuric acid was added, the volume was raised to 1L and then filtered. Indigo carmine was kept in brown bottle till the experiment completed. The blank test was carried out by titration of a mixture of 25ml Indigo carmine solution and 750ml of (dd H2O**).** All were analyzed in duplicates.

Phytate

Phytic acid content of the samples was estimated using the method compiled by Jain and Mogra (2006). One gram of moisture free finely ground sample was taken in a conical flask and added 50 ml HCl. The mixture was shaken in a shaker for 3 hours and filtered. The clear filtrate thus obtained was reduced to 25 ml over water bath. The filtrate was neutralized adding required amount of sodium hydroxide. Ten ml of 0.01 per cent ferric chloride was then added and the mixture heated over water bath for 15 minutes, cooled to room temperature and filtered again using a pre-weighed filter paper. The residue was washed with ethanol and then ether.

Results and Discussion

Chemical properties of buckwheat whole (BW), buckwheat groats (BG) and buckwheat husk (BH) were analyzed and the results obtained on dry matter basis have been presented in following sections (Table 1-3).

Proximate analysis

Moisture, crude fat, ash, crude protein, crude fibre, carbohydrates and energy contents of BW, BG, and BH were estimated and results are depicted in Table 1.

The chemical analysis of buckwheat fractions for proximate composition revealed significant difference for moisture, fat, ash, protein, fibre and energy. Moisture content was significantly ($p \leq 0.05$) higher in BW (8.56g/100g) followed by BG (7.19g/100g)

and BH (5.16g/100g). Highest amount of crude fat content was exhibited in BG (2.68g/100g) followed by BW (2.02g/100g) and BH (0.76g/100g). Ikeda and Yamashita (1994) reported that seeds of common buckwheat contain 1.5-3.7% total lipids. The highest concentration is in embryo and the lowest in the hull at 0.4-0.9%. Groats or dehulled seeds of buckwheat contain 2.1-2.6% total lipids. Total ash was significantly higher in BW (2.34g/100g) than BG (2.04g/100g) and BH (2.15g/100g). Bonafaccia *et al.,* (2003) studied the composition and technological properties of the flour and bran from common and tartary buckwheat. The content of ash was found between the range of 1.82-4.08% among grain, brain and flour. Protein, the body building nutrient, was significantly higher in BG (14.88g/100g) than BW (11.34g/100g) and BH (9.91g/100g). Fornal (1999) reported that buckwheat flour contains from 8.5% to near 19% of proteins depending on the variety, pesticides used and fertilization that are likely to affect the total concentration of buckwheat proteins.

BW and BH showed significantly higher content of crude fibre (9.35g/100g and 10.74g/100g) respectively than in BG (3.46g/100g). It was found that crude fibre was highest in bran (10.74 g/100g) and was lowest in BG (3.46 g/100g) as buckwheat groats was dehusked form of grain which affects the fibre content. Bonafaccia and Kreft (1994) found from 3.4% to 5.2% of total dietary fibre in buckwheat samples and products. Buckwheat may have, because of its fibre content may have an important role in prevention and treatment of hypercholestremia (He *et al.,* 1995). It was observed that all three fractions of buckwheat exhibited almost similar values of carbohydrate content which ranged from 66.35 g/100g in BW to 71.25 g/100g in BH.

In the whole grain of buckwheat, starch content varies from 59% to 70% of the dry mass, demonstrating fluctuations under variable climate and cultivation conditions. However the difference was found to be statistically non-significant. The energy values can also be seen to be varying possibly due to protein and carbohydrate content among BW, BG and BH. The values ranged from 329 kcal in BW to 362 kcal in BG.

Kim *et al.,* 2004) reported that buckwheat grains contain a variety of nutrients, the main compounds being protein, dietary fibre, lipids and carbohydrate. The total content of components depends on the variety or environmental factors (Barta *et al.,* 2004). It can be concluded that BG is rich in protein, fat and content as compared to BW and BH. Pomeranz and Robbins (1972) also suggested that BG is a good protein supplement.

Mineral profile

The major mineral contents for BW, BG and BH are presented in Table 2. The significant difference was found between flours for calcium, Iron, and Zinc. In case of calcium, BH recorded higher value 149.66 ppm than BW and BG (76.80 ppm and 38.13 ppm). Buckwheat is rich in potassium (k), magnesium (Mg) calcium (ca) and Sodium (Na) (Wei *et al.,* 1995) and most of minerals are concentrated mainly in bran (Bonafacia *et al.,* 2003).

Iron content was significantly higher in BW (106.83 ppm) followed by BG (80.61 ppm) and BH (47.10 ppm). Among three flours zinc content was found significantly higher in BG (23.83 ppm) than BW (20.50 ppm) and BH (14.83 ppm). Bonafacia *et al.,* (2003) studied the content of Se, Zn, Fe, Co, Ni were analyzed in the flour and bran of common and tartary buckwheat. There is relatively small difference in the content of Iron, and

chromium between flour and bran fractions. Though there was no significant difference observed for copper among BW, BG, and BH but the copper content of BW (14.1567 ppm) was found slightly higher than BG (10.9367 ppm) and BH (11.8333). Ikeda (1994) analyzed the content of zinc, copper and manganese in various samples of buckwheat. Generally the content of minerals in buckwheat grains and their morphological fractions (dry basis) reaches (6: 2-2.5% in whole grains, 1.8-2.0% in kernel, 2.2-3.5% in dehulled grains, about 0.9% in flour, and 3.4-4.2% in hulls (Li and Zhang, 2001).

Table.1 Proximate analysis of buckwheat whole (BW), buckwheat groats (BG) and buckwheat husk (BH)

S.N.	Treatment	Nutrients g/100g													
		Moisture		Fat		Ash		Protein		Fibre		CHO		Energy (Kcal)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	BW	8.56	0.62	2.02	0.42	2.34	0.03	11.34	0.05	9.35	1.18	66.35	1.48	329	8.11
2	BG	7.19	0.45	2.68	0.22	2.04	0.02	14.88	1.31	3.46	0.45	69.72	1.89	362	3.79
3	BH	5.16	0.62	0.76	0.34	2.15	0.04	9.91	0.91	10.74	1.00	71.25	2.50	331	4.99
	GM	6.97	1.56	1.82	0.89	2.18	0.13	12.05	2.35	7.85	3.44	69.11	2.77	341	16.98
	Se	0.32		0.19		0.02		0.53		0.54		1.1586		3.41	
	CD5%	1.13*		0.68*		0.07*		1.84*		1.87*		4.00NS		11.83*	
	CD1%	1.72*		1.03*		0.11*		2.79*		2.83*		6.07NS		17.92*	
	CV	8.18		18.66		1.76		7.67		11.94		2.90		1.74	
	Treatment	8.81		2.85		0.06		19.61		44.80		18.80		1048.26	
	Error	0.32		0.116		0.00		0.85		0.87		4.026		35.06	

GM=General Mean, * Significant at 5% and 1% level of significance, NS = Non-significant

Table.2 Mineral composition of buckwheat whole (BW), buckwheat groats (BG) and buckwheat husk (BH)

S.N.	Treatment	Calcium (ppm)		Iron (ppm)		Zinc (ppm)		Copper (ppm)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	BW	76.80	0.57	106.83	2.68	20.50	1.58	14.15	3.55
2	BG	38.13	0.97	80.61	5.00	23.83	1.28	10.93	0.58
3	BH	149.66	6.12	77.10	2.73	14.83	1.17	11.83	1.06
	GM	88.20	49.14	88.18	14.41	19.72	4.11	12.30	2.36
	Se	2.07		2.10		0.78		1.25	
	CD5%	7.18*		7.27*		2.71*		4.3342	NS
	CD1%	10.88*		11.01*		4.11*		6.5659	NS
	CV	4.08		4.13		6.90		17.62	
	Treatment	9621.44		791.87		62.11		8.28	
	Error	12.93		13.25		1.85		4.70	

GM=General Mean, * significant at 5% and 1% level of significance, NS = Non-significant

Table.3 Anti- nutritional analysis of buckwheat whole (BW), buckwheat groats (BG) and buckwheat huck (BH)

S.N.	Teatment	Tannin%		Phytic acid%	
		Mean	SD	Mean	SD
1	BW	4.16	-0.00	18.36	1.90
2	BG	4.16	-0.00	6.23	2.40
3	BH	5.54	0.00	18.30	3.71
	GM	4.61	0.69	14.30	6.51
	Se	0.00		1.60	
	CD5%	0.00	NS	5.55	
	CD1%	0.00	NS	8.41	
	CV	0.00		19.45	
	Treatment	1.91		146.41**	
	Error	0		7.73	

GM=General Mean, *significant at 5% and 1% level of significance, NS = Non-significant

Plate.1

(Buckwheat-BW) (Buckwheat Groats-BG) (Buckwheat Husk-BH)

Anti-nutritional analysis

The anti-nutritional factors viz tannin and phytic acid was analyzed in all flour fractions. The results obtained are presented in Table 3 and discussed below.

Tannin content was found to be highest in BH (5.54%) than BW (4.16%) and BG (4.16%). No significant difference was found in the content of tannin. Sharma and Sahgal (1992) reported that buckwheat seeds contain from 0.5 to 4.5% tannin depending on the genotype and on ecological factors. The phytic acid content was significantly ($p \leq 0.05$) lower in BG (6.233%) than BW (18.36%) and BH (18.30%). Skrabanjia *et al.,* (2004) studied nutrient content in buckwheat milling fractions. A unique distribution was found in for phytate as correlation was significantly positive in husk, bran and semolina fractions, while correlation is significantly negative in flour fractions.

Depending on chemical analysis of buckwheat whole (BW), buckwheat groats

(BG) and buckwheat husk (BH), the buckwheat groats considered nutritionally dense due to its better macro and micronutrient and low anti-nutritional content than BW and BH. Pomranz and Rabbins (1972) determined protein content and amino acid composition in buckwheat and found that groat is a good protein supplement. Phytic acid was found significantly negative in flour fraction than husk, bran and semolina fractions (Skrabanja *et al.,)*

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