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Effect of Soaking and Germination on Nutritional profile and Antinutrients of Buckwheat Whole (*Fagopyrum esculentum*)

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A B S T R A C T

Buckwheat (*Fagopyrum esculentum*) is a broad-leafed herbaceous annual. It belongs to the family Polygonaceae, which is generally referred to as the buckwheat. Hindi name for buckwheat is "Kutu". Its consumption may be less as it is hard to digest containing anti nutritional factors. Looking to its nutritional and therapeutic significance soaking and germination methods applied to observe the effects on nutritional and antinutritional profile of buckwheat whole (*Fagopyrum esculentum*).The study was conducted at Department of Food & Nutrition, College of Home science, Maharana Pratap University of Agriculture & Technology Udaipur, Rajasthan, India. Buckwheat whole (BW) processed as soaking and germination for 6, 12, 18 hr and 24, 36, 48 hr respectively and subjected for chemical analysis (proximate, minerals, anti-nutrients) to find out the effect of processing on anti-nutrients with nutritional profile. Protein content was found highest in germination for 24 hr followed by unprocessed buckwheat whole. Calcium, zinc and copper content of buckwheat whole were found higher after germination as compared to unprocessed buckwheat whole. Tannin content was lower on soaking for 18 hr and 12hr in comparison to unprocessed buckwheat whole. A continuous degradation was observed in phytic acid with soaking and germination.

Introduction

Common buckwheat (*Fagopyrum esculentum*) is a broad-leafed herbaceous annual. It belongs to the family Polygonaceae, which is generally referred to as the buckwheat, rhubarb or sorrel family. However, because its seed structurally and chemically resembles the cereal grains, buckwheat is usually handled and classed with the cereals. 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. Hindi name for buckwheat (*Fagopyrumesculentum*) 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. It is also sporadically cultivated in the Nilgiri and PaIani hills of southern India mainly as a green manure crop.

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 to provide various health benefits much sought after in natural foods. 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)**.**

Despite the balanced amino acid composition, the buckwheat protein digestibility in humans and in animals is relatively low because of anti-nutritional factors present in common buckwheat, including protease inhibitors (such as trypsin inhibitors) and tannins (Ikeda et al1991). Germination of buckwheat seeds significantly reduces the activity of proteases inhibitors, so seedlings and young buckwheat plants as a food source show improved digestibility and utilization of proteins. Its consumption may be less as it is hard to digest containing anti nutritional factors such as protease inhibitor, tannin, phytic acid. There are number of technologies identified by which anti -nutritional activity can be diminished or reduced to a large extent. Looking to its nutritional and therapeutic significance soaking and germination methods applied to observe the effects on nutritional and antinutritional profile of buckwheat whole (Fagopyrumesculentum).

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) was 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.

Soaking

Two hundred g sample of BW was cleaned, weighed and soaked in 200ml distilled water for 6, 12 and 18 hr. After six hr. water was drained out. The amount of drained water was measured. Sample was weighed separately just after removal of water after spreaded on aluminum foil and dried in oven at 600C for 5 hours. Sample (BW) weighed during drying. Sample was ground in a mixer separately and weighed again and packed in aluminum foil and stored in desiccators for chemical analysis.

Germination

Buckwheat whole was germinated separately for 24, 36 and48 hr.(Plate 1). 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:

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

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:

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

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:

Fat (g/100g) =

$$\frac{\text{Weight of ether extract fat (B-A)}}{\text{Weight of sample (gm)}} \text{ X100}$$

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:

Ash (g/100g)=

$$\frac{\text{Weight of ash (g)}}{\text{Weight of sample taken (g)}} \text{ X100}$$

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 antinutritional 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 deionised water (dd H2O**)** and kept for 4 hours at room temperature and filtered in volumetric flask with filterpaper. 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

Effect of soaking and germination treatments on chemical composition as proximate analysis, mineral profile and anti nutrients of buckwheat whole are presented in. Table 1 to 3.

Proximate composition of processed and unprocessed buckwheat whole (BW) is presented in Table 1.Significant difference was found in moisture content among soaking and germination treatments which ranges from 7.71 to 11.46 g/100g. The moisture content was found highest in 18 hr Soaking (B4:11.46 g/100g) indicating that with increasing soaking time the moisture content increases. Abdulsalami *et al.,* (2010) investigated the effect of processing on the proximate and mineral composition of Bambara groundnut and found an increase in moisture content. Crude fat content of unprocessed buckwheat whole was found higher (B1:2.03 g/100g) than processed buckwheat whole and there was slight decrease in fat content with soaking (B2 to B4) and germination (B5 to B7). Ocheme (2008) studied the effects of soaking and germination on some physico-chemical properties, of millet flour and sensory properties of porridges. It was reported that fat, decreased significantly as result of soaking and germination. The lower fat content of the germinated samples can be due to the breakdown of lipids that occurs during germination in order to obtain the energy required for the plant's development (Urbano *et al.,* 2005). There was significant difference in ash content in buckwheat whole after processing (B2 to B7). A slight decrease in ash content was also observed on soaking (B2to B4). Abdulsalami *et al.,* (2010) also found slight decrease in ash content from 5.37 to 2.89 (g/100 dry wt) after processing methods. No significant difference was observed in the protein content of buckwheat whole after processing (B2 to B7). On soaking protein content was found to reduce (P>0.05) but it increased germination. Muyanja and Kikafunda (2003) reported that increased protein content in germinated flour from non-germinated sorghum flour might be due to improved protein extractability and attributed to microbial protease activity, breakdown of tannin and phytates which are known to bind protein. Fibre content was decreased gradually on soaking and germination, (B2 – B7) as compare to unprocessed buckwheat whole (B1). Abdulsalami *et al.,* (2010) also reported a decrease in fibre content after processing

A significant difference in carbohydrate content was observed after processing of buckwheat whole. The highest carbohydrate content was found on 36 hr Germination of buckwheat whole (B6:70.50g/100g).On germination and soaking of buckwheat whole carbohydrate content was found to enhance as compared to unprocessed buckwheat (B1). This may be due to increase in content of starch on soaking and germination. Qian and Kuhn (1999) also reported that starch is the major content of buckwheat varies from 59.70% and accumulated in endosperm. It may be possible that while soaking and germination it moves towards outer layer. There was a significant difference in energy content among all processed flours. Energy content ranged between 320 kcal to 338 kcal respectively.

The major mineral content as calcium, iron, zinc, and copper in buckwheat whole after processing is presented in Table 2.There was a significant difference in calcium content of buckwheat whole (B1) after processing (B2-B7) and was found higher than unprocessed buckwheat whole (B1). Zinc content of buckwheat was found higher after germination (B2 – B7) as compared to unprocessed buckwheat whole (B1). Saikia *et al.,* (1999) measured phytic acid, tannin and trypsin inhibitor activity and found that phytic acid

lowers the availability of P, Zn, and calcium and other minerals. Processing techniques have been found to reduce significantly. The level of phytate and tannin (Ahmed *et al.*, 2006). So, it can be said that the higher content of calcium and zinc after processing of buckwheat whole was because of decreased phytate and tannin.

Iron content of processed buckwheat whole was found slightly higher in 6 hr soaking (B2:147.53 ppm) and germination for 24 hr (B5:117.77) as compare to unprocessed buckwheat whole (B1:106.83 ppm). The iron content was found slightly lower in over soaking (12 hr, 18 hr) and germination (36 hr, 48 hr) as compare to unprocessed flour. Saharan *et al.*, (2001) studied the effects of cooking method on Ca, Fe, and P. It was reported that soaking and sprouting reduced the content of these minerals slightly, probably due to leaching into the soaking medium.

The copper content of buckwheat whole was found to increase with soaking duration of 6 hr,12hr and 18 hr (B2, B3, B4) and germination 24 hr,36hr and 48hr (B5, B6, B7) as compare to unprocessed buckwheat whole (B1). Saharan *et al.*, (2001) reported that inexpensive and simple processing treatments had significant positive in part on in vitro availability of the minerals, most likely due to a reduction in anti-nutrients as phytic acid. The Findings of anti-nutrients as tannin and phytic acid in buckwheat whole after processing are reported in Table 3.

Though there was no significant difference found in tannin content of buck wheat whole after processing but a slight decrease was observed and was lowest in soaking for 18hr (B4: 3.46%) as compared to unprocessed buckwheat whole (B1:4.16%). Doss *et al.*, (2011) studied the effects of processing at different methods. Like soaking, cooking and autoclaving on the content of anti-nutritional compounds and found that soaking and cooking decrease the levels of tannins.

Table.1 Effect of soaking and germination on proximate analysis of buck wheat whole (BW)

Flour	Proce-ssing	Nutrients (g/100g)													
		Moisture		Fat		Ash		Protein		Fiber		CHO		Energy (Kcal)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BW	B1	8.57	0.62	2.03	0.42	2.35	0.04	11.35	0.06	9.35	1.19	66.36	1.49	329	8.11
	B2	7.71	0.51	1.95	0.52	2.17	0.10	10.95	0.00	9.70	1.05	67.54	0.89	331	7.98
	B3	7.84	0.93	1.77	0.11	2.17	0.03	10.51	0.44	7.69	0.54	70.02	1.29	338	5.98
	B4	11.46	0.61	1.86	0.10	2.19	0.00	10.95	0.44	8.53	0.18	65.01	1.07	320	3.48
	B5	7.85	0.15	1.29	0.53	2.58	0.13	12.99	0.25	8.23	0.11	67.06	0.70	330	5.85
	B6	7.98	0.15	0.76	0.74	2.52	0.14	12.11	0.51	6.13	1.68	70.50	1.53	337	8.66
	B7	8.52	0.38	0.91	0.44	2.35	0.07	11.82	0.76	5.97	1.38	70.43	1.36	337	8.12
	GM	8.56	1.33	1.51	0.63	2.33	0.18	11.52	0.88	7.94	1.64	68.13	2.32	332	840
	SE	0.36		0.24		0.04		0.33		0.49		0.84		3.50	
	CD5	1.06*		0.70NS		0.13*		0.97NS		1.44*		2.44*		10.14*	
	%														
	CD1	1.44*		0.94NS		0.18*		1.32NS		1.94*		3.30*		13.69*	
	CV	7.05		20.62		3.67		4.40		16.63		2.14		1.763	

BW= Buckwheat whole, B1= No processing, B2=6 hr Soaking, B3=12 hr Soaking, B4=18 hr Soaking, B5=24 hr Germination, B6= 36 hr Germination, B7= 48 hr Germination, GM= General Mean, * significant at 5% and 1% level of significance, NS= Non-significant

Table.2 Effect of soaking and germination on mineral composition of buckwheat(BW)

Flour		Calcium (ppm)		Iron (ppm)		Zinc (ppm)		Copper (ppm)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
BW	B1	76.81	0.58	106.83	2.68	20.50	1.59	14.16	3.55
	B2	160.70	1.65	147.53	2.90	20.18	1.50	23.13	2.91
	B3	184.05	12.56	100.15	2.75	19.78	0.88	30.86	0.72
	B4	101.65	1.50	86.03	6.16	21.88	1.03	15.75	3.40
	B5	108.00	1.08	117.77	1.60	23.18	1.01	17.39	0.71
	B6	104.46	1.18	69.69	3.87	21.01	1.16	16.70	1.51
	B7	98.53	1.33	62.91	3.17	33.68	1.05	21.76	1.06
	GM	119.17	36.57	98.69	27.83	22.89	4.75	19.96	5.81
	SE	2.86		1.83		1.00		1.42	
	CD5%	8.30*		5.32*		2.90*		4.12*	
	CD1%	11.22*		7.19*		3.92*		5.56*	
	CV	4.36		3.52		6.67		14.41	

BW=Buckwheat whole, B1= No processing, B2=6 Soaking, B3=12 hr Soaking, B4=18 hr Soaking, B5=24 hr, Germination, B6=36 hr, Germination, B7=48 hr Germination, GM=General Mean, * significant at 5% and 1% level of significance

Table.3 Effect of soaking and germination on anti-nutrients of buckwheat whole (BW)

Flour		Tannin%		Phytic Acid%	
		Mean	SD	Mean	SD
BW	B1	4.16	0.00	18.37	1.90
	B2	4.16	0.00	14.83	1.33
	B3	3.46	0.00	18.67	4.10
	B4	3.46	0.00	17.50	3.41
	B5	4.16	0.00	16.60	0.46
	B6	4.16	0.00	14.77	1.18
	B7	4.16	0.00	18.87	3.09
	GM	3.96	0.32	17.09	2.69
	SE	0		1.21	
	CD5%	0NS		3.52 NS	
	CD1%	0NS		4.75 NS	
	CV	0		16.43	

B1= No processing, B2=6 hr Soaking, B3=12 hr Soaking, B4=18 hr Soaking, B5=24 hr Germination, B6=36 hr Germination, B7=48 hr Germination, GM= General Mean, NS= Non-significant

Plate.1

(Buckwheat) (24h germination) (36h germination) (48h germination)

As compared to unprocessed buckwheat whole (B1) a continuous degradation was observed in phytic acid after processing (B2 to B7). It shows that germination for long duration is not beneficial. Shimelis and Rakshit (2007) also obtained anotable reduction (over 75%) in phytic acid in three kidney bean varieties after germination.

Phytic acid contents were reduced only with germination treatment (42.6%) while the other treatments did not bring about any large reduction although all the tested anti nutritional factors were significantly reduced with different processing techniques, tannins proved to be the most labile, while phytic acid was the most resistant to all processes except sprouting. **(**Yasmin 2008). Processing techniques as soaking, cooking, germination and fermentation have been found to reduce significantly the level of phytate and tannin by exogenous and endogenous enzyme formed during processing. Germination of seeds decreases tannin and phytic acid contents of the guar gum seeds with decrease in albumin fraction (Ahmed *et al.,* 2006).

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