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SOME PHYSICO-CHEMICAL PROPERTIES OF GERMINATED AND UNGERMINATED BUCK WHEAT (*FAGOPYRUMESCULENTUMDUR*)

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Abstract: The present work was undertaken to study the physico-chemical properties of germination and ungerminated of buckwheat (*Fagopyrumesculentumdur*). During germination the nutritional value of buckwheat grains increased. The moisture content of ungerminated and germinated flour samples of buckwheat varied between 11.4 % to 12.1 % (wb) and increased after germination. Whereas, Ash content of above flour sample of buckwheat varied between 2.3 % to 1.9 % (wb) and decreased after germination. The total carbohydrate, total sugar, reducing and non-reducing sugar contents of flour sample of buckwheat varied between 71.23% to 61.21%, 2.40% to 4.85%, 0.80% to 0.97% and 1.60% to 3.49% respectively. The protein content of above flour sample of buckwheat varied between 12.4 % to 13.1% and increased after germination. The starch content decrease after germination, this is might be due to increased α-amylase and β-amylase activities. The amylase and amylopectin content increased after germination, this is might be due to increase in gelatinization rate. WAI (water absorption index) and WSI (water solubility index) of ungerminated and germinated flour sample of buckwheat varied between 109.3 % to 150.1 % and 14.5 % to 24.8 % respectively increased after germination. OAC (Oil Absorption Capacity) and viscosity decrease after germination. Buckwheat flours colour increased after germination. The phytic acid (antinutritional factor) content of ungerminated and germinated flour samples of buckwheat varied between 15.65 % to 13.42 % and decreased after germination.

Keywords: Buckwheat, Germination, Antinutritional factor.

1. Introduction

Buckwheat is neither a nut nor a cereal like wheat, but rather a pseudocereal, whose history dates back over 1000 years. In India two species of buckwheat are cultivated in the Himalayas (*F.esculentum*and *F. tataricum*). It is highly nutritive, unlike cereals which are deficient in lysine, one of the essential amino acids for human health (Anonymous, 1979). The flour of buckwheat has various kinds of vitamins such as B_1, B_2 and niacin at relatively high level (Pomernaz, 1983). Dehulled buckwheat groats were found to contain 75% starch, 13.9% proteins and lipids. The total dietary fiber (TDF) in groats may range from 5 to 11%.

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Bran fraction obtained by milling of buckwheat is especially rich in dietary fibre (13-16%) which is considerably less in its flour (1.7-8.5%). Majority of its starch is readily digestible, while a small portion (4-7%) resists hydrolysis (Mazza, 1993). In comparison with other cereals such as rice, wheat flour or corn, buckwheat contains higher levels of zinc, copper and manganese (Steadman *et al.,* 2001). The bio-availability of zinc, copper, and potassium from buckwheat is especially high (De Francischi *et al.,* 1994). These minerals play an essential role in the prevention of hypertension and anaemia. Buckwheat contains many flavonoid compounds which are known in reducing the blood cholesterol, keeping capillaries and arteries strong and flexible and assisting in prevention of high blood pressure.

Malting is a controlled germination process which activates the enzymes of the resting grain, resulting on conversion of cereal starch to fermentable sugars, practical hydrolysis of cereal proteins and other macromolecules. The greatest use of malt in beer manufacturing is the process of malting which consists of controlled germination and subsequent drying of feed. Barley is most common material for malting. Malt production is second largest use of barley and is largest food application of these grains. The uses of malt include preparation of breakfast cereals, malted milk concentrate, infant foods, bakery products and candies. Malting process allows partial digestion of stack and protein. Viscosity of the product can be reduced to any desired level depending on the extent of germination. The goal of malting is to produce high enzyme activity and characteristic flavour.

2. Material and Method

2.1 Material

Preparation of raw material

Buckwheat was procured from the local market of Sangrur, Punjab, India. The grains were cleaned by application of aspirator & sieving for the removal of foreign seed, saw, dust, etc. Cleaned grains was washed and steeped in excess water for 12 hr. The water was changed once or twice during the steeping period. At the end of steeping period the grains were again washed and kept for germination. Steeped grains was spread on moist cloth to about 2 – 3" thick bed, then covered with another moist cloth. During germination, water sprinkled to keep the sprouts moist. After germination, the sprouts were dried in cabinet drier at 65 ± 2^0C for 5 – 6 hrs and remove the sprouts. Grinding of the green malt was done using a burr mill. Malt flour was sieved through 52 mesh size to make it a homogeneous mixture and stored at room temperature.The chemicals used in these study, were obtained from the Merck India Ltd.

2.2 Methods

2.2.1 Proximate Composition Analysis

Germinated and ungeminated buckwheatflour moisture, protein, fat, carbohydrate and ash content were determined. Moisture was determined gravimetrically in hot air oven at 105±5°C for 3 hrs and then to constant weight. Protein was estimated by digestion and distillation (Kjeldahl) method, fat content by solvent (n-hexane) extraction, total carbohydrate content was estimated by AOAC, 1995 method and ash content by muffer furnace method (550±10°C for 5 hrs).

Amylose content was estimated by spectrophotometric method (spectrophotometer I D 5000 HACH, USA) as described by Scott *et al* (1998).Water binding capacity (WBC) of the flour of germinated and ungerminated buckwheat was estimated using the method described by Yamazaki, (1953).Oil binding capacity (OBC) of flours of germinated and ungerminated buckwheat was estimated by the method described by Yamazaki, (1953).

2.2.2 Starch content

The reagents used for the starch estimation were anthrone reagent (dissolved 200mg anthrone in 100ml of ice cold 95% sulphuric acid (H_2SO_4)), 80% ethanol, 52% perchloric acid, Standard glucose solution (stock 100mg glucose dissolved in 100ml water, working standard 10ml of stock diluted to 100ml with distilled water).

0.1gm (d/b) sample was taken in a test tube with hot 80% ethanol to remove the sugar. Then the sample was centrifuged and the residue was washed repeatedly with 80% ethanol till the washing did not give the color with anthrone reagent and sample was placed for drying. After drying 5ml water and 6.5ml, 52% perchloric acid were added and extracted for 20 min at 0°C. The solution was centrifuged and the resultant residue was washed repeatedly with fresh perchloric acid and centrifuged. All supernatant was collected and volume made up to 100ml. One ml supernatant was taken and made up to volume to 1ml with water. Standard was made by taking 0.2, 0.4, 0.6, 0.8, 1ml of the working standard and made up the volume to 1ml with distilled water. Then 4ml of anthrone reagent was added and placed into a boiling water bath for 8 min. After rapid cooling, the sample was placed for determination of absorbance at 630nm.

$$Amount\ of\ starch\ in\ 100mg\ of\ the\ sample\ = \frac{mg\ of\ glucose}{volume\ of\ test\ sample} \times 100$$

2.2.3 Reducing sugar content

The reagents used for reducing sugar estimation were Dinitrosalicylic Acid Reagent (DNS Reagent) -Dissolve by stirring 1 g dinitrosalicylic acid, 200 mg crystalline phenol and 50 mg sodium sulphite in 100 ml 1% NaOH. Store at 4°C. Since the reagent deteriorates due to sodium sulphite, if long storage is required, sodium sulphite may be added at the time of use. 40% Rochelle salt solution (Potassium sodium tartrate).

Weigh 100 mg of the sample and extract the sugars with hot 80% ethanol twice (5 mL each time). Collect the supernatant and evaporate it by keeping it on a water bath at 80°C. Add 10 ml water and dissolve the sugars. Pipette out 0.5 to 3 ml of the extract in test tubes and equalize the volume to 3 ml with water in all the tubes. Add 3 ml of DNS reagent. Heat the contents in a boiling water bath for 5 min. When the contents of the tubes are still warm, add 1 ml of 40% Rochelle salt solution. Cool and read the intensity of dark red color at 510 nm. Run a series of standards using glucose (0–500 µg) and plot a graph.

Calculate the amount of reducing sugars present in the sample using the standard graph.

2.2.4 Estimation of Crude Fibre

The reagent used were Sulphuric acid solution (0.255 ± 0.005 N): 1.25 g concentrated sulphuric acid diluted to 100 ml (concentration must be checked by titration). Sodium hydroxide solution (0.313 ± 0.005 N): 1.25 g sodium hydroxide in 100 ml distilled water (concentration must be checked by titration with standard acid). Extract 2g of ground material with ether or petroleum ether to remove fat (Initial boiling temperature 35–38°C and final temperature 52°C). If fat content is below 1%, extraction may be omitted. After extraction with ether boil 2 g of dried material with 200 ml of sulphuric acid for 30 min with bumping chips. Filter through muslin and wash with boiling water until washings are no longer acidic. Boil with 200 ml of sodium hydroxide solution for 30 min. Filter through muslin cloth again and wash with 25 ml of boiling 1.25% H2SO4, three 50 ml portions of water and 25 ml alcohol. Remove the residue and transfer to ashing dish (pre-weighed dish W_1). Dry the residue for 2 h at 130 ± 2°C. Cool the dish in a desiccator and weigh (W_2). Ignite for 30 min at 600 ± 15°C. Cool in a desiccator and reweigh (W_3).

$$Crude\ fibre\ in\ ground\ sample = \frac{Loss\ in\ weight\ on\ ignition\ (W_2 - W_1)(W_3 - W_1)}{weight\ of\ the\ sample} \times 100$$

2.2.5 Water Solubility Index (WSI)

The supernatant from the water absorption index determination was decanted into pre-weighed moisture dish and evaporated to constant weight in a precision oven at 103℃. Water solubility index (WSI) was calculated as the percent weight of the original sample weight recovered (Iwe, 1998).

$$WSI\ (\%) = \frac{Weight\ of\ dry\ solid\ in\ supernatent}{Dry\ weight\ of\ sample} X\ 100$$

2.2.6 Colour determination by Lovibond Tintometer

The method determines the colour of flours by comparison with Lovibond glasses of known colour characteristics. The color is expressed as the sum total of the yellow and red slides used to match the colour of the sample in a cell of the specified size. The cell was previously cleaned with carbon tetrachloride and allowed to dry. The cell filled with sample was placed in Lovibond Tintometer. The color was expressed as

$$Colour\ reading = (a\ Y + 5\ b\ R)\ or\ (a\ Y + 10\ b\ R)$$

Where, a = sum total of the various yellow slides (Y) used

b = sum total of the various red (R) slides used

Y + 5R is the mode of expressing the colour of light coloured samples; and

Y + 10 R is for the dark-coloured samples

2.2.7 Viscosity of flours

The Brookfield rotational viscometer (Model LVT2, Brookfield Engineering Lab, Stoughton, Mass, USA) has been used to analyze rheology of pastes, colloidal suspensions and solutions (Sikdar and Ore, 1979). Flow properties of different flours were determined according to a standard method of ISI-17-Ie. Apparent viscosity (μ_a) of flours of corn, germinated and ungerminated grains of buckwheat was determined using Brookfield viscometer with spindle No.1 at room temperature at different rpm (6, 12, 30 and 60). Flour samples were taken about 200ml in 250ml beaker and apparent viscosity (μ_a) in mPa·s was calculated by multiplying the dial reading at different rpm (6, 12, 30 and 60) of the Brookfield viscometer with the factors (10, 5, 2, and 1),respectively, as described in the manual of LVT Brookfield viscometer.

2.2.8Phytic acid content of buckwheat flour

The reagent used were 3% Trichloro acetic acid (TCA), 3% Sodium sulphate in 3% TCA, 1.5N NaOH, 3.2N HNO_3, $FeCl_3$ solution: Dissolve 583mg $FeCl_3$, in 100ml of 3% TCA, 1.5M Potassium thiocynate (KCSN): Dissolve 29.1g in 200ml water, Standard $Fe(NO_3)_3$ solution:

Weigh accurately 433 mg $Fe(NO_3)_3$ and dissolve in 100 ml distilled water in a volumetric flask. Dilute 2.5 ml of this stock standard and make up to 250 ml in a volumetric flask. Pipette out 2.5, 5, 10, 15 and 20 ml of this working standard into a series of 100 ml volumetric flasks and proceed from step 16. Weigh flours contain 5 to 30 mg phytate p into a 125 ml flask. Extract in 50 ml 3% TCA for 30 min with mechanical shaking or with occasional swirling by hand for 45 min. Centrifuge the suspension and transfer a 10 ml aliquot of the supernatant to a 40 ml conical centrifuge tube. Add 4 ml of $FeCl_3$ solution to the aliquot by blowing rapidly from the pipette. Heat the contents in a boiling water bath for 45 min. If supernatant is not clear after 30 min, add one or two drops of 3% sodium sulphatein 3% TCA and continue heating. Centrifuge (10-15 min) and carefully decant the clear supernatant. Wash the precipitate twice by dispersing well in 20 to 25 ml 3% TCA, heal in boiling water for 5 to 10 min and centrifuge. Repeat washing with water. Disperse the precipitate in a few ml of water and add 3 ml of 1.5N NaOH with mixing. Bring volume to approximately 30 ml with water and heat in boiling water for 30 min. Filter hot (quantitatively) through a moderately retentive paper No.2. Wash the precipitate with 60-70 ml hot water and discard filtrate. Dissolve the precipitate from the paper with 40 ml hot 3.2 N HNO_3 into a 100ml volumetric flask. Wash with several portions of water, collecting the washing in the same flask. Cool the contents to room temperature and dilute to volume with water. Transfer a 5ml aliquot to another 100ml volumetric flask and dilute to approximately 70 ml. Add 20ml of 1.5M KSCN, dilute to volume, and read colour immediately (within 1 min) at 480 nm. Run a reagent blank with each set of samples. Find out the fig iron present in the test from the standard curve, and calculate the phytate P as per the equation:

$$Phytate\ P\ (mg) = \frac{mg\ Fe\ x\ 15}{weight\ of\ sample\ (g)}$$

3. Resultand Discussion

The moisture content of ungerminated and germinated flour samples of buckwheat varied between 11.4 % to 12.1 % (wb) and increased after germination. This finding is similar to the results reported by Khatoon and Prakash, (2006) in germinated legumes.

Ash content of above flour sample of buckwheat varied between 2.3 % to 1.9 % (wb) and decreased after germination. This result is completely in agreement with result noted by Tatsadjieu *et al.* (2004). The decreased in ash content might be due to loss of minerals and rootlets.

The total carbohydrate, total sugar, reducing and non-reducing sugar contents of flour sample of buckwheat varied between 71.23% to 61.21%, 2.40% to 4.85%,0.80% to 0.97% and 1.60% to 3.49% respectively. This finding is similar to the result reported by (Srivastva *et al.,* 1988; Dogra *et al.,* 2001 ; Bewley and Black, 1994).The decrease in carbohydrate content might be due to active respiration process during soaking and germination. On the other hand, total sugar, reducing and non-reducing sugar contents increased after germination, this is might be due to increase the activities of α-amylase and β-amylase enzymes.

The protein content of mentioned flour sample of buckwheat varied between 12.4 % to 13.1% and increased after germination. This finding is similar to the results reported by Tatsadjieu*et al.* (2004).

The fat contents of flour sample of buckwheat varied between 3.8% to 1.8 % and decreased after germination. This finding is similar to the results reported by (Kaukovirta*et al.,* 1993; Diboforie*t al.,* 1994).The decreased in fat content might be due to increased activity of lipase enzyme.

Table 1: Chemical composition of ungerminated and germinated buckwheat flour

Sample → / (%) ↓	**Ungerminated buckwheat flour**	**Germinated buckwheat flour**
Moisture	11.4	12.1
Ash	2.30	1.9
Carbohydrate	71.23	61.21
Total Sugar	2.40	4.85
Reducing sugar	0.80	0.97
Non-reducing sugar	1.60	3.49
Fat	3.8	1.8
Protein	12.4	13.1
Starch	55.8	51.56
Amylose	16.74	18.35
Amylopectin	37.96	34.67
Crude fiber	7.8	9.74

The starch, amylase and amylopectin contents of flour sample of buckwheat varied between 55.8% to 51.6%, 16.47% to 18.35%, and 39.06% to 34.67% respectively. The starch content decrease after germination, this is might be due to increased α-amylase and β-amylase activities. This finding is similar to the results reported by Sharma *et al.* (2007). The amylase and amylopectin content increased after germination, this is might be due to increase in gelatinization rate. This finding is similar to the results reported by Palmer, (1989).

Crude fibre content of above flour sample of buckwheat varied between 7.8 % to 9.74 % and increased after germination. This finding is similar to the results reported by Muyanja *et al.*(2001) in germinated millet grains.

WAI (water absorption index) of ungerminated and germinated flour sample of buckwheat varied between 109.3 % to 150.1 % and increased after germination. This result is in agreement with the results obtained by Cira-Chavez *et al.* (2009). The increase in water absorption index might be due to increased protein content during germination.

WSI (water solubility index) of ungerminated and germinated flour sample of buckwheat varied between 14.5 % to 24.8 % and increased after germination. This result is completely in agreement with the result obtained by Pelembe *et al.* (2000). The increase in water solubility index might be due to carbohydrate content decreased as a result of hydrolysis by the amylase enzymes.

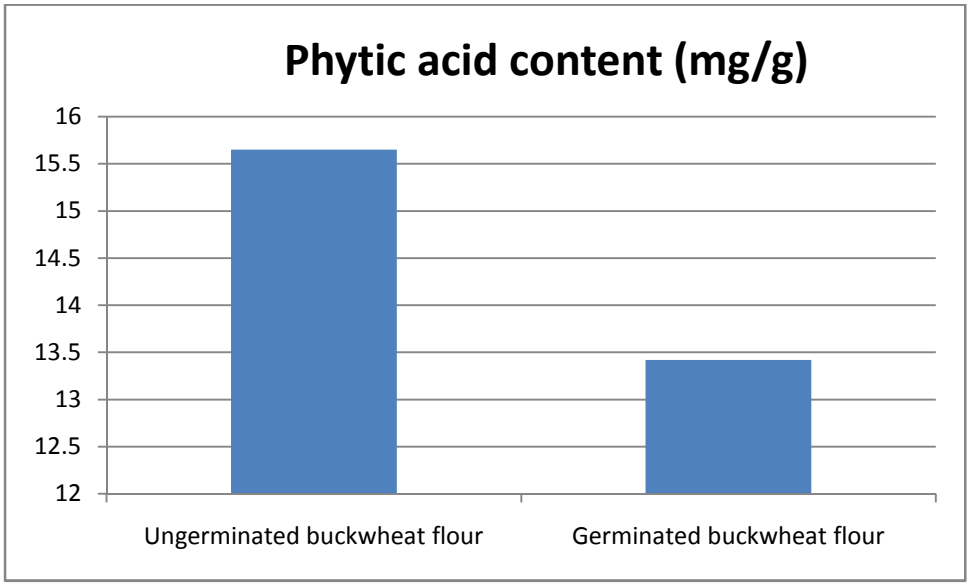
OAC (Oil Absorption Capacity) of flours sample of buckwheat varied between 1.55 to 1.21 and decrease after germination.

Viscosity determination of ungerminated and germinated flour sample of buckwheat varied between 35 cpu to 22.5 cpu, and decreased after germination. This result is completely in agreement with the result obtained by Zeeman et al. (2007). The decreased in viscosity might be due to the action on the starch by hydrolyzing enzymes that were produced during germination.

Colour of flours of ungerminated and germinated flour sample of buckwheat varied between 7 to 7.1, and increased after germination. The colour of flours were measured by using Lovibond tintometer and reading are indicated in tcu (total colour units).

Table 2 Physical properties of ungerminated and germinated flours of buckwheat

Samples		**Buckwheat(ungerminated)**	**Buckwheat (germinated)**
WAI (%)		109.3	150.1
WSI (%)		14.5	24.8
OAC (%)		1.55	1.21
Viscosity (cpu)		35	22.5
Colour	**(Yellow)**	1.6	1.7
	(Red)	1.1	1.4
	(Blue)	0.8	1.1
	(TCU)	6.6	7.2



Graph 1: Phytic acid content of buckwheat flour after germination

The phytic acid content of ungerminated and germinated flour samples of buckwheat varied between15.65 % to 13.42 % and decreased after germination. This finding is similar to the results reported by Bilgicli, (2008).

5. Conclusion

The buckwheat flour further utilize as fasting purpose and for make extruded product etc because the germinated buckwheat flour gives higher nutritional value and less antinutritional factor phytic acid content, higher crude fiber increase the digestibility and reduces the chronical diseases.

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