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Nutritional and functional quality analysis and amino acid score evaluation of germinated wheat (*Triticum aestivum*) grain

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Abstract
In the present study wheat (major staple crop across the globe) was sprouted (germinated) to analyze its impact on nutritional attributes like macronutrients (protein, sugar, carbohydrates and fats), micronutrients (folic acid, dietary fibers), trace elements (calcium, iron, magnesium, phosphorus, sodium and zinc), antinutritional factors (trypsin inhibitor, phytic acid, tannins, polyphenols, oxalates), amino acid profile and biological value. The nutrients analysis revealed an enhancement in proteins and sugars, folic acid and dietary fibers during sprouting. The amino acid profile analysis revealed an enhancement in essential amino acids (EAAs) and significant improvement in essential amino acid index (EAAI), protein efficiency ratio (PER), biological value (BV) and nutritional index (NI). Except tyrosine all the amino acids were in positive correlation with each parameter studied viz. EAAI, PER, BV and NI. The amino acid score evaluation as per FAO/WHO standards had shown a significant improvement, as a result of increase in EAAs during sprouting.

Keywords: Antinutritional factors; amino acid profile; biological value; amino acid score, grain morphology; PER; EAAI; Nutritional index; Pearson correlation

Introduction
From a long period cereals have been used as a major nutrition source for human. Of them wheat is one of its kind to produce variety of products. For the majority of population wheat is the major dietary source for energy and nutrition in developing countries which are fed directly to traditional diet based on cereal and starch crops. However most of the population is consuming the food product without knowing its nutritional quality. Cereal grains although rich in nutrition contains certain antinutritional factors, which limits their digestibility and bioavailability of minerals. According to Dewey and Brown [1] few efforts have been made at household level to increase the nutrient density of food. The nutritional quality of cereal thus can be improved using various methods including fortification of limiting nutrients. The traditional and natural processes for improvement of nutrient availability and improvement involve soaking, germination and fermentation [2].

The primary objective of germination (germination) is the development of hydrolytic enzymes that are inactive in raw seeds [3]. Germination of grains is the better alternative as the technique is simple, inexpensive as compared to fermentation and improves availability of certain nutrients. Improvement of digestibility is one of the prominent reasons to incorporate the technique for household utilization of grains for infant foods. Germination is considered as the hydrothermal treatment given to seeds under ambient conditions which lead to synthesis of new compounds with higher nutritional value and stability of grain [4]. There is a decrease in the caloric content of sprouted seed and thus comparatively the nutrient to energy ratio is higher than original seed, therefore sprouted legumes and cereals are now the part of health enthusiasts.

Sprouts production is a simple process of germination under controlled conditions and can be achieved in any season and serves good alternative to vegetables [5]. A wide variation in chemical, thermal, pasting and textural properties of seeds, flour and starches of black gram, chickpea lines and kidney bean on germination has been reported previously [6]. Breakdown of antinutritional factors, bioavailability of minerals and enhancement of free sugars, vitamins and protein have been reported previously [7]. Thus the present work was aimed at to carry out a detailed qualitative and quantity analysis to understand the possible effect of germination (germination) on seed characteristics, composition and nutritional indices.

Material and methods
Materials
The wheat variety (PBW-550) was procured from Punjab Agriculture University (PAU), Ludhiana, Punjab (India). Grains were 2-3 months old in viable condition and were not treated chemically. A pre weighed sample of grains were cleaned, dried and sorted before milling. The milled flour was sieved through 60 mesh sieves before analysis. Germination of grains was carried out after soaking for 6-8 hours, under controlled temperature and humidity conditions in a seed germinator. Germinated grains were dried at 60 °C for 6-8 hours, milled and sieved through 60 mesh sieve. The resultant raw and germinated flour was stored in air tight containers at 4 °C until analyzed.

Chemical composition
The compositional analysis of the both germinated and un-germinated wheat flour samples were carried out in triplicates for moisture, crude protein (Kjeldahl method), crude fat (solvent extraction), crude fiber, ash and dietary fiber as per AOAC [8].

Trace element analysis
The standard method described by Association of Official Analytical Chemists was used for mineral content analysis of

the samples [8]. The samples were ashed at 550 °C. The ash was boiled with 10 ml of 20% hydrochloric acid in a beaker and then filtered into a 100 ml standard flask. This was made up to the mark with deionized water. The minerals were determined from the resulting solution. Sodium [Na] was determined using the standard flame emission photometer using NaCl as the standards [8]. Phosphorus was determined calorimetrically using KH_2PO_4 as the standard. Calcium [Ca], Magnesium [Mg] and Iron [Fe] were determined using Atomic Absorption Spectrophotometer. All values were expressed in mg/100 g.

Antinutritional factors

The *trypsin inhibition activity (TIA)* was assayed in terms of inhibition of bovine trypsin on the substrate benzoyl-DL-arginine-pnitrianilide (BAPNA) hydrochloric [9]. Tannin contents were determined by the modified vanillin-HCl methods [10]. A standard curve was prepared using catechin after correcting for blank and tannin concentration was expressed in mg/100 g.

The *Oxalate content* was determined by AOAC [8] method. The concentration of oxalate in each sample was obtained from the calculation:

1 ml 0.1 permanganate = 0.006303 g oxalate.

The *tannins* were estimated using Vanillin-HCl method [10]. A defatted seed material was extracted for tannin in acidic methanol and prepared vanillin-HCl reagent was added to develop color. Catechin standards were run along with the sample and OD was measured at 500nm. Results were expressed in mg/100g dry weight.

The *phytic acid* was estimated by the method of Davies and Reid [11]. The content was extracted with nitric acid and reacted with ferric ammonium sulphate in a boiling water bath. On cooling isoamyl alcohol and ammonia solution was added and centrifuged at 3000 rpm for 10 min. The alcoholic layer was separated and color was measured at 465nm with amyl alcohol taken as blank. The results were expressed as mg phytic acid/100g dry weight.

The *polyphenols* were estimated using Folin-Denis method [8]. Defatted sample was extracted using 1% HCl in methanol and content was refluxed for 2 h. Volume was made up to 100ml with water and 0.2ml extract was taken, to this 0.5ml Folin-denis reagent was added and mixed with saturated sodium carbonate again volume was made to 10 ml with water. The OD was taken at 760 nm after 30 min. The results were calculated as mg tannic acid equivalent/g sample and expressed as mg/100g on dry weight basis.

Amino acid analysis

All samples were analyzed using the physiological kits for gas chromatography–flame ionization detection (Phenomenex, USA). The grain samples were milled to flour (60 mesh sizes) and then hydrolyzed with concentrated HCl. The analysis was performed, as directed in the kit's manual. The GC column used was the ZB-AAA GC column, which was provided in the kits and standard analysis conditions were used, as described in the kit's manual.

Protein quality evaluation

The nutritional value and protein quality evaluation was done according to the methods of Chavan *et al*. [12]. Essential amino acid index (EAAI) was calculated using the method of Oser *et al*. [13].

$$EAAI = \sqrt{\frac{Lys_a \times Try_a \times \ldots.\times His_a}{Lys_b \times Try_a \times \ldots.\times His_b}}$$

Where "a" is the amino acid in test sample and "b" is the amino acid in reference protein sample.

Protein efficiency ratios (PERs) on the basis of interaction between Leucine-prolein, and Leucine-tyrosine were calculated using the modified regression equations as described by Mune-Mune *et al*. [14].

PER-1= -0.684+0.456(leu)-0.047(pro)
PER-2= -0.468+0.454(leu)-0.105(tyr)

The amino acid score for infants (pre-school) and adults were calculated as the ratio of observed value to the reference pattern as provided by FAO/WHO.

Morphological characteristics

The microstructure analysis of both raw as well as germinated wheat flours were carried out by scanning electron microscope (SEM), JEOL, Tokyo, Japan, Model No. JSM 6610-LV at magnification of 1500-2500 X. Flour samples were mounted on aluminum stub using a double backed cellophane tape, coated in auto fine coater, JEOL-JFC-1600, with gold palladium (60:40, g/g).

Statistical analyses

The analysis was carried out in replicates for all analysis. The mean and standard deviation were calculated. Coefficient of variation was calculated by observing the ratio of Standard deviation to mean of both germinated and un-germinated analysis. The data were further subjected to analysis of variance (ANOVA). A multiple comparison procedure of the treatment means was performed by Duncan's new multiple range test (Duncan, 1955). The correlation coefficients were computed using SPSS 16. Significance of the differences was defined as $P \leq 0.05$.

Results and Discussion

Chemical analysis

A significant statistical difference was observed between the compositions of raw and germinated grains as a result of germination with respect to protein, ash and total sugar contents (Table-1). The germinated wheat flour had a significant (25%) higher protein as compared to raw wheat flour. Various studies on different crops observed a similar finding [15] which show that germination is the only traditional method that results an increase in protein content. These changes were attributed to an increase in the proteases activity, which results an increase in the amino acids [16]. The germinated wheat flour had shown an increment in the total sugars content (43.28%) due to the significant increase in the non-reducing and reducing sugars. This increment in the sugar content is attributed to the utilization of food reserve carbohydrate due to enhanced enzymatic action (alpha-amylase activity) which results in the degradation of carbohydrates into simpler compounds like simple sugars and oligosaccharides [17].

The ash content and ether extract did not show any significant change however a slight increase in the ether content had been

observed. The germinated tiger nut seed flour showed a similar finding for ash content [18]. The slight increase in ether extract might be due to non-conversion of free fatty acids to carbohydrates which may lead to increase in fat composition during germination [19].

The germinated wheat flour had higher crude fiber content relative to raw wheat flour. A similar finding has been observed by Chauhan *et al*. [20] in amaranth grains. Functional components like dietary fibers and folic acid also had shown a significant increase after Germination. The previously reported studies for some pulses by Sibian *et al*. [21] in Bengal gram had indicated the similar observation.

Effect of germination on trace element availability

In the plants the bioavailability of minerals depends on the presence or absence of various antinutritional factors. The reduction in the phytic acid and other antinutritional factor after germination results in the increase in the availability of minerals to some extent [22] observed an increase in the HCL-extractability of calcium, iron and zinc in pearl millet after germination. The

Wheat flour was analyzed for trace elements like calcium, iron, magnesium, phosphorus, sodium and zinc as shown in Table-1. The calcium and sodium had shown a small increment during Germination. A significant increase in the iron and phosphorus was observed in germinated flour, however, no significant increase in the magnesium and zinc was found in germinated wheat flour. A similar finding has been observed in Faba beans by Eskin and Wiebe [23]. The Mbithi-Mwikya *et al*. [24] relates the post germination increase in the trace elements to the decrease in the phytate contents.

Table 1: Chemical characteristics of un-germinated and germinated wheat

Constituents (%)	Wheat (un-germinated)	Wheat (Germinated)	Coefficient of Variation (%)
Carbohydrates	75.64±0.02^{a}	68.46±0.45^{b}	0.070
Protein	11.13±0.12^{b}	13.92±0.08^{a}	0.086
Ash	1.07±0.02^{a}	1.06±0.02^{ab}	0.007
Sugar	5.29±0.02^{b}	7.58±0.04^{a}	0.252
Non-reducing sugar	4.45±0.03^{b}	6.35±0.04^{a}	0.248
Reducing sugar	0.85±0.01^{b}	1.23±0.09^{a}	0.264
Lipids	1.05±0.01^{ab}	1.10±0.03^{a}	0.189
Crude fiber	2.69±0.02^{b}	3.98±0.04^{a}	0.345
Dietary Fibers	4.32±0.04^{a}	6.60±0.05^{a}	0.296
Folic Acid (mcg/100g)	70.41±0.32^{a}	81.12±0.12^{a}	0.100
Trace-elements (mg/100g)			
Calcium	47.60±0.01^{ab}	48.21±0.04^{a}	0.005
Iron	4.22±0.04^{b}	4.71±0.02^{a}	0.051
Magnesium	92.55±0.14^{ab}	93.24±0.07^{a}	0.003
Phosphorus	65.91±0.02^{b}	72.54±0.03^{a}	0.045
Sodium	1.23±0.03^{b}	1.35±0.04^{a}	0.443
Zinc	3.82±0.08^{ab}	3.87±0.12^{a}	0.006

Results are mean±SD of three independent determinations and expressed as (g/100g). Values in a row with different letters are significantly different (P<0.05).

Effect of germination on anti-nutritional factors

The anti-nutritional factors limit the bio-availability of nutrients to human diet. Their mode of action depends on the type of functional groups present in their molecules e.g. the phosphate groups of phytic acid (inositol hexakisphosphate) which form stable complexes with cations (like Fe^{2+}, Ca^{2+}, Mg^{2+} and Zn^{2+}), thus preventing their bioavailability. Antinutritional profile of raw and germinated wheat is shown in Table-2. During the process of Germination the amount of antinutritional factor decreased significantly. The decrease in the tannins, polyphenols and phytic acid is attributed to some extent by soaking before germination. The trypsin inhibitors and oxalates get reduced during the metabolic stage of germination due to activation of some catabolic enzymes. The i*n-vitro* protein digestibility and availability of free amino acids are the function of trypsin activity as per the observation of Mbithi-Mwikya *et al*. [24]. A 43.51% decrease in the trypsin inhibitor activity was found in germinated wheat flour as compared to raw flour in the present study.

Table 2: Antinutritional factors in wheat as affected by germination

Sample	Trypsin Inhibitor activity (TIU)	Phytic Acid (mg/100g)	Tannins (mg/100g)	Polyphenols (mg/100g)	Oxalates (mg/100g)
Wheat					
Un-germinated	150.05±0.07^{a}	0.65±0.04^{a}	0.22±0.06^{a}	0.92±0.03^{a}	4.69±0.04^{a}
Germinated	84.75±0.11^{b}	0.32±0.03^{b}	0.06±0.02^{b}	0.89±0.07^{b}	2.34±0.09^{a}
Variability (%)	43.51	50.77	72.73	3.26	50.11

Results are mean±SD of three independent determinations. Values in a column with different letters are significantly different (P<0.05).

The tannins have an effect on the IVPD and also inhibit the digestive enzymes [25]. There was an approximately 50% decrease in the phytic acid content. The soaking of grains prior to germination causes the loss of phytic acid content [26].

Results obtained in present study had shown no significant variability in polyphenol contents; however some researcher reported a significant decrease in polyphenols [27] but in some cases significant increase in the polyphenol content during germination has also been observed [28]. The oxalates are the anti-nutritional components of plants which causes the blockage of renal tubules by calcium oxalate crystals and development of urinary calculi [29]. Oxalates, like phytates, bind trace calcium and magnesium and interfere with their metabolism. A significant reduction in oxalate content was observed in germinated wheat in the present study.

Effect of germination on amino acid profile

The wheat was analyzed for amino acid profile. The other parameters like essential amino acid index (EAAI), nutritional index, biological value and protein efficiency ratio were calculated using amino acid profile. The germination activates proteases, which helps in metabolizing proteins thereby increasing nutrient bioavailability [30] and improves amino acid profile. The values of non-essential amino acids for wheat varied from (alanine-3.97±0.01; aspartic acid-5.84±0.04; glutamic acid-25.22±0.14; glycine-3.89±0.03; prolein-7.36±0.05; serine-5.76±0.02 and tyrosine-2.54±0.02 g/100g protein) for un-germinated flour to (alanine-4.88±0.02; aspartic acid-8.05±0.05; glutamic acid-19.6±0.12; glycine-4.02±0.03; prolein-4.39±0.07; serine-4.24±0.02 and tyrosine-2.92±0.01 g/100g protein) for germinated flour.

During the germination a significant decrease in glutamic acid, proline and serine content was observed. The total essential amino acids are reported in Table-3 with histidine and without histidine. Increase in the total amino acid was observed in germinated sample. Total amino acids enlisted under essential amino acids are Isoleucine, leucine, lysine, methionine, cystine, arginine, phenylalanine, threonine, valine and histidine. The corresponding values for enlisted essential amino acid ranged from 3.25±0.02 to 3.53±0.02; 4.13±0.01 to 4.39±0.04; 1.99±0.02 to 2.19±0.02; 2.22±0.01 to 2.86±0.02; 0.91±0.01 to 0.99±0.01; 5.07±0.01 to 5.46±0.04; 2.27±0.03 to 2.86±0.02; 1.92±0.02 to 2.15±0.02; 3.22±0.02 to 3.71±0.03 and 1.83±0.05 to 2.09±0.03 (g/100g protein) in respective order. Cereals are slightly deficient in lysine, but increase in the lysine content after germination had been observed in wheat due to germination.

Due to significant improvement in the overall amino acid profile there is improvement in the EAAI, Biological value, Nutrition Index and protein efficiency ratios (PER). The similar results have been reported previously in Bengal gram flour after germination by Sibian *et al.* [21]. The ratio of essential amino acid to total amino acid had shown a slight increase as a result of germination, which indicates that, the proportion of essential amino acid increased during germination, as a result of increment in the free amino acid. The protein-based food material is of good nutritional quality when its biological values is between 70 to 100% and essential amino acid index (EAAI) is above 90%, to be useful when the values is around 80% and to be inadequate when below 70% [13]. As these values were not up to the mark as discussed above, therefore, these cereals cannot be considered for protein efficient diet. However during the process of germination the values got closer to 70%, which means controlled germination process improves the protein quality and nutritional value.

Table 3: Amino acid profile of un-germinated and germinated wheat grain

Protein Profile	Wheat (Un-germinated)	Wheat (Germinated)
Total non-essential amino acid	67.05±0.24^{b}	61.48±0.22^{d}
Total essential amino acid (with Histidine)	32.95±0.03^{b}	38.52±0.11^{a}
Total essential amino acid without Histidine)	30.69±0.12^{b}	35.85±0.08^{a}
Total Aromatic Amino acid	5.92±0.11^{e}	7.38±0.19^{c}
Total Acidic amino acid	38.15±0.23^{a}	35.34±0.24^{c}
Total Basic Amino Acid	10.94±0.14^{e}	12.46±0.17^{b}
Leucine/Isoleucine ratio	1.27±0.05^{c}	1.24±0.06^{c}
Total Essential Amino Acid/Total Amino acid (%age)	32.95±0.03^{b}	38.52±0.11^{a}
Essential Amino Acid Index (%)	67.99±0.33^{d}	76.55±0.30^{b}
Biological value	62.41±0.22^{f}	71.74±0.11^{e}
Nutritional Index (%)	7.56±0.16^{c}	10.65±0.14^{a}
PER-1	0.85±0.01^{f}	1.11±0.01^{d}
PER-2	1.14±0.01^{e}	1.22±0.01^{d}

Results are mean±SD of three independent determinations and expressed as (g/100g). Values in a row (for respective cereal) with different letters are significantly different (P<0.05). (-) symbol indicates the decrease in the value.

The cysteine in wheat showed a positive correlation with all parameters, but the tyrosine showed the least correlation with nutritional value. The Pearson correlation coefficient was calculated to observe the extent of relationship between essential amino acid and components of amino acid profile (Table-4). Arginine had shown a higher correlation coefficient with PER-1 and biological value. Except tyrosine all other amino acids were in positive correlation with each parameter. The obvious reason for this correlation value was the change in the amino acid content in wheat after germination. The tyrosine content had shown a slight decline after germination. The limiting amino acid like lysine also had shown a positive correlation coefficient, which might be due to significant increment in lysine during germination.

Table 4: Pearson correlation coefficient between essential amino acids and nutritional quality parameters of amino acid profile

Characteristics	Lysine	Leucine	Isoleucine	Valine	Argenine	Methionine	Cysteine	Threonine	Phenylalanine	Tyrosine	Histidine
Essential Amino Acid Index	.955*	.924	.982*	.998**	.988*	.988*	.933	.976*	.911	-.967*	.954*
Protein Efficiency Ratio-1	.955*	.953*	.995**	.988*	1.000**	.994**	.975*	.997**	.964*	-.913	.990*
Protein Efficiency Ratio-2	.953*	.938	.990**	.997**	.998**	.993**	.957*	.992**	.945	-.939	.976*
Biological Value	.954*	.954*	.995**	.987*	1.000**	.994**	.976*	.997**	.966*	-.910	.991**
Nutritional Index	.883	.878	.956*	.983*	.978*	.957*	.927	.990**	.961*	-.893	.966*

Effect of germination on amino acid score

The amino acid scores for infants and adults are shown in Table-5 analyzed according to essential amino acid (g amino acid/100g protein) pattern of FAO/WHO [31] standard protein references values. Due to increase in the overall amino acid quality as a result of germination there was an increase in the amino acid score as well. In the case of pre-school/Infants, the amino acid score enhanced for Methionine+Cysteine followed by Valine in germinated wheat, whereas lowest change was observed for Phenylalanine+Tyrosine. For the adults, a considerable increase in amino acid score for Methionine, Methionine+Cysteine and Valine was observed. Only Cysteine showed a decrease in the values. The lysine had been found as the first limiting amino acid however small increment was also reported. The variation in essential amino acid indices and calculated biological values were due to changes in the values of essential amino acids during germination.

Table 5: Amino acid score for Infants and adults

Amino Acid Score (for infants/pre-school- 1-2 yrs)	FAO/WHO(2007)	Wheat	
		Raw	Germinated
Isoleucine	3.1	104.83	114.05
Leucine	6.3	65.49	69.71
Lysine	5.2	38.25	42.15
Methionine + Cysteine	2.6	120.18	143.70
Phenylalanine + Tyrosine	4.6	104.73	125.59
Threonine	2.7	71.10	80.03
Valine	4.2	76.60	88.41
Amino Acid Score (for adults)	**FAO/WHO(2007)**	**Wheat**	
		Raw	**Germinated**
Isoleucine	3	108.32	117.85
Leucine	5.9	69.93	74.44
Lysine	4.5	44.21	48.71
Methionine	1.6	138.48	171.59
Cystine	0.6	151.48	165.14
Methionine + Cysetine	2.2	142.03	169.83
Phenylalanine + Tyrosine	3.8	126.78	152.03
Threonine	2.3	83.46	93.95
Valine	3.9	82.50	95.21
Histidine	1.5	122.57	139.27

Effect of germination on grain morphology

Germination affects the quality of starch, as hydrolytic enzymes viz. alpha-amylase is activated [32] and hydrolyses starch and non-starch polysaccharides. Morphological characteristics of germinated wheat flour starch were observed using SEM (scanning electron microscope). The structural differences between raw Fig. 1a and germinated Figs. (1b-1f) wheat flours as observed. Raw starch granules were round, non-porous and slightly covered with protein matrix. Conversely, starch granules had been partially digested and the protein matrix was more associated in germinated samples. Partially digested starch had shown distorted structure probably, highly porous, cluster orientation due to high enzymatic susceptibility [32]. The porous structures were also reported in the scanning electron micrograph of starch granules in triticale and barley by Lorenz *et al*. [33].

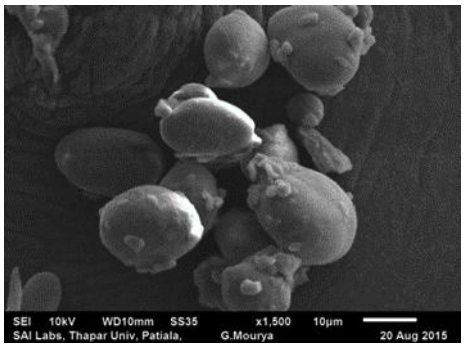

Fig 1a: Granule compact with well-defined structure

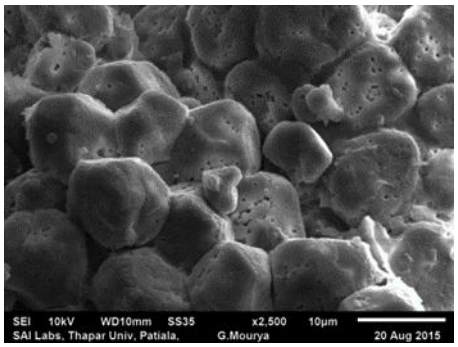

Fig 1b: Porous granules with structural disintegration

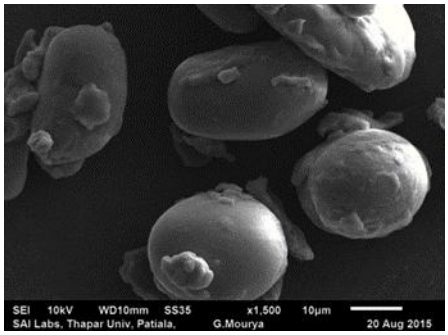

Fig 1c: Less clustering and or orientation

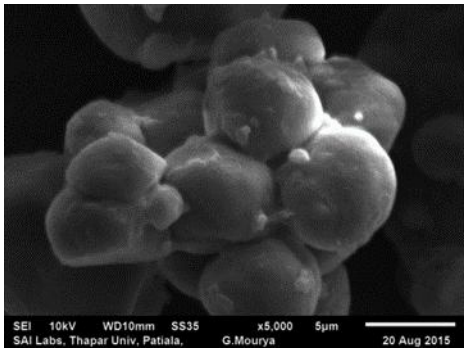

Fig 1d: Granules clustering and agglomeration

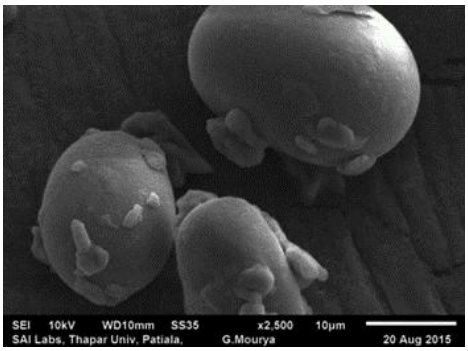

Fig 1e: Granule round with less protein matrix

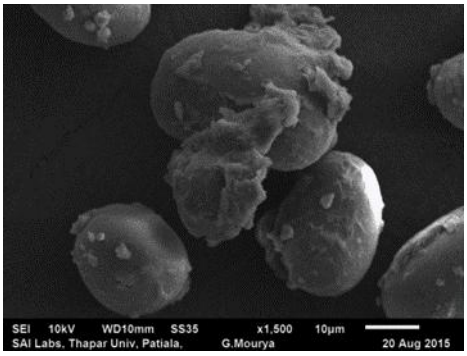

Fig 1f: Granule more associated with protein matrix with surface distortion

Photographs indicating the effect of germination on granular characteristics

Fig 1: Scanning electron micrographs of un-germinated (1a) and germinated (1b-1f) wheat flour samples (at 1500 X resolution)

Conclusion

The outcome of the present studies indicates that germination is an effective process to improve the nutritional value of wheat. Improvement in the nutritional profile and decrease in the anti-nutritional factors shows the benefit of germination. Germination enhanced the amount of total protein, crude fiber, dietary fiber, and folic acid to a significant level. Antinutritional factors like phytic acids, tannins and oxalates decreased to a considerable amount during germination, which in turn enhanced the extractability of trace elements or divalent ions. Amino acid profile also improved during germination and therefore helps to improve the protein quality and nutritional value of wheat. Components associated with amino acids like EAAI, nutritional index and biological value improved due to enhancement of essential amino acids. Pearson correlation coefficient depicts the association of essential amino acid and amino acid profile. Microstructure of flour represented a clear reference to damaged starch and enhancement of protein matrix after germination. Germination therefore is the potential process to improve the overall nutritional quality and to reduce the anti-nutritional factors.

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