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Characterization of germinated and un-germinated (*Fagopyrum esculentum*) buckwheat grain and its flour

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Abstract
In recent years, buckwheat flour has gained much importance due to increased interest of researchers towards natural origin food than chemical modified food as modification induces some unwanted changes. Therefore, the different properties of buckwheat are of great significance so that it could be incorporated in multiple food system to increase functionality. In the light of above benefits, a study was conducted to understand the effect of germination on morphological, thermal and pasting properties of buckwheat flour derived from germinated and non-germinated grains. Results revealed germination caused a significant decrease in different viscosities *viz.*, peak, trough, breakdown, setback and final viscosity. It also caused the reduction in gelatinization temperature of starch in buckwheat flour measured using Differential scanning calorimetry (DSC) when heated at constant rate. In addition to this loss in birefringence of starch molecule due to breakdown of amylopectin which is responsible for crystallinity with a large decrease in gelatinization energy. Thus, crystalline starch was changed to amorphous form in germinated grain. The swelling capacity of flour derived from germinated grains was found to be significantly increased ($p<0.05$). Scanning electron microscopy (SEM) showed smooth surface and dense packing of starch in non-germinated grain which got modified after germination.

Keywords: buckwheat flour, differential scanning calorimetry, scanning electron microscopy

1. Introduction

Buckwheat (*Fagopyrum Esculentum)* is a pseudo cereal in which interest of researchers has seems to be increased due to its nutritive and health benefit effects (Christa & Soral-Śmietana, 2008) [6]. The major polysaccharide in buckwheat grain is starch which ranges from 59-70% (d. b.). The functionality of starch depends upon amylose and amylopectin. The arrangement of starch into sphero-crystalline granule gives swelling property which depends upon the temperature of water (Araujo-Farro *et al*., 2014) [3]. The main components includes proteins, polysaccharides, fibre, lipids, rutin, polyphenols, micro and macro elements (Kim *et al*., 2004; Wronkowska *et al.*, 2010) [12, 23]. The protein content of buckwheat grain is of high biological value (Kato *et al*., 2001) [10]. The other positive aspects are acts as antioxidant, reduce hypertension, lower blood cholesterol and prevention of diabetes which makes it unique among grains and clearly justifies, why this food grain should be given more attention? (Jacquemart *et al*., 2012) [9]. Most of the study conducted in past were only limited to science of rheology for individual and no comparative study was conducted between germinated and ungerminated buckwheat grain focusing gelatinization temperature and viscosity at different temperatures. Hence, in this study an attempt has been taken to fill the gap of knowledge how the germinated and non-germinated grain and flour differs in characteristics.

Work done by researchers describes thermal processing on aqueous buckwheat flour (starch) causes gelatinization. Gelatinization is the thermal breakdown of crystalline structures and reforming in native starch granules which causes an event of changes such as swelling of the granules and leaching of amylose (Atwell *et al*., 1998; Zhou *et al*. 2009) [4, 24]. The aqueous solution when heated causes the intra-molecular interaction between hydroxyl group and hemi-acetal oxygen group of amylose linkage *i.e.*, 1-4 α-D glucopyranosyl. Not only this, but also the intermolecular bonding occurs between -OH group attached to second carbon of glucose moiety and O-6 of D-glucopyranosyl of other glucose moiety forming amylose granule (Tako, 2014) [20].

With continued heating, a temperature will be reached at which the hydrogen bonds are sufficiently weakened so that water can be absorbed by the granules where upon they begin to swell tangentially and simultaneously lose their characteristic. This combined effect of interaction which causes the swelling and absorption of water molecule by hydrogen and van deer wall bonding is called as 'gelatinization' (Araujo-Farro *et al*., 2014) [3]. The disintegrated starch granules give a dispersion of amylose, amylopectin and granule fragments which also causes the loss of birefringence. This newly formed structure which is notoriously unstable and goes through cooling effect that causes a decrease in kinetic energy. The lower energy causes association of bonds between –OH groups of sixth carbon atom and hemiacetal oxygen or hydrogen of D-glucopyranosyl. The methyl group finally provide stability and elasticity property to starch granule. The association of linear amylose molecules give rise to "setback" and leads to thick and viscous matrix called as 'retrogradation' (Tester & Morrison, 1990; Araujo-Farro *et al*., 2014) [21, 3]. This finally depicts about the energy content and viscosity of flour before and after heat and water treatment. Thus germination has been considered a convenient process to improve functional and nutritional properties of grain.

2. Materials and Methods

2.1 Materials

The freshly harvested buckwheat grains of variety *Fagopyrum Esculentum* were procured from Chamoli, Uttarakhand, India. To remove the impurities the grains were screened through 60-mesh and were given dry and wet cleaning before use.

2.2 Methods

Germination of grain was carried out according to method described by Hara (2007) [7]. Grains were soaked in 0.1% formalin (H.CHO) solution in the ratio 1:2 at 25°C for 4h. Soaked grains were washed and germinated in moist muslin cloth with continuously supply of water through capillary in a seed germinator (Macro Scientific works Pvt. Ltd. India) at 25°C for 24 h and 95% RH. Germinated grains were dried in tray drier at 50°C for 5-6 h and then milled to obtain fine flour. The prepared flour was subjected to 60 mesh screen to remove coarse particles and stored in polyethylene bags at 4°C until used for further analysis.

2.3 Morphological properties

Scanning electron microscope (SEM) (Hitachi Model S-800) was used to observe the morphology of buckwheat grain as well as the starch fractions in flour obtained from germinated and ungerminated grain. In sample preparation for observing shape and size, the grain was stained over a solution of osmium trioxide (OsO_4) for 12 h which acts as a controlled oxidising agent. The gas was completely removed by maintaining the negative pressure and using sodium hydroxide (NaOH) as desiccant. During testing samples were placed on the platform coated with Pt-Pd (Platinum-Palladium) metal for 4 min and the equipment was electrified at 10 kV potential difference. The properties were determined according to method given by Qian & Khun (1999) [16].

2.4 Thermal properties

DSC thermograms were obtained for germinated and the ungerminated buckwheat flour. Sample was prepared by thoroughly mixing of flour with distilled water in the ratio 1:3.5 (w/w) to make homogenous slurry. Each sample of about 9g was weighed correctly to 0.1 mg and transferred to a pre-weighed aluminium dish sealed hermetically using encapsulation press and weighed again. An empty aluminium dish was used as a reference. The samples were subjected to testing within half an hour after preparation. The parameters studied were gelatinization temperature (°C), onset temperature (T_o), peak temperature (T_p), end set temperature (T_e) and enthalpy (ΔH). The methodology adopted was similar to Inglet (2009) [8].

2.5. Pasting properties

A rapid visco analyser (RVA) (Model 3D, RVA; Newport Scientific Pvt. Ltd., Warrie Wood, Australia) was used to determine the pasting properties of flour. The sample was prepared by mixing 3g flour measured correctly to 0.1mg in 25 ml of distilled water (least count 0.1ml) to have final moisture content of 13% and 11% in the slurry obtained from germinated and ungerminated buckwheat flour respectively. The slurry was applied controlled heating and cooling in cyclic manner under a constant shear rate. During heating, first the sample was kept at a temperature of 50°C for 60 seconds then heated to raise the temperature from 50°C to 95°C such that 12°C increase in temperature within 60 seconds and further held at 95°C for 300 seconds. The observations were recorded in terms of pasting temperature (PT), peak viscosity (PV), hot paste viscosity (HPV) (viscosity at the end of holding time at 95°C), cold paste viscosity (CPV) (viscosity at the end of the hold time at 50°C) according to method described by Qian & Kuhn (1999) [16].

2.6. Statistical analysis

All the tests were performed in triplicate. Data was subjected to one way analysis of variance (ANOVA). STATISTICA software (ver. 16.0) was used for the analysis of data. Means were compared using Duncan multiple comparison test according to Wójtowicz *et al*. (2013) [22].

3. Results and Discussion

3.1 Effect of germination on morphological properties of buckwheat flour

Micrographs at resolution 50X obtained using SEM revealed that ungerminated grain had achenes with three edged shape. The embryonic axis runs from the bottom of kernel to the top of grain while embryo was located in pointed part of the kernel. The grain was composed of outer pericarp called hull. Next to hull laid the inner pericarp which covered the seed coat called Testa. Further next to it laid two cotyledons whose lateral side was attached to endosperm which is a rich source of starch, protein and oil content Figure. 1 (a). The observations were in agreement to the findings reported by Pomeranz & Sachs (1972) [15]; Inglett *et al*. (2009) [8].

Germinated grain possessed the radical (white) and embryonic root which grew downward towards Earth called as geotropism. The root possesses the hairs which increases the ability to soak water and nutrients from its surrounding Figure. 1 (b). The seed coat (testa) and leaves were in brown and green in colour respectively. Throughout germination the continuous proteinaceous layer covering starch granules got degraded which was responsible for rough texture of grain. This breakdown caused oozing out of starch from granule thus increasing swelling property of starch. It was accompanied by transformation and breakdown of dense starch granule packing to loosen it with voids formation (free space). It was in agreement with the report by Sindhu & Khatkar (2016) [19] who observed Morphological properties of common buckwheat (*Fagopyrum Esculentum Moench*) flour. The breakdown of starch polymer into mono and disaccharides caused a significant increase in glucose and maltose content. The difference in the structure of flour particles derived from ungerminated and germinated grains is shown in Figure. 1 (c) and Figure. 1 (d) respectively.

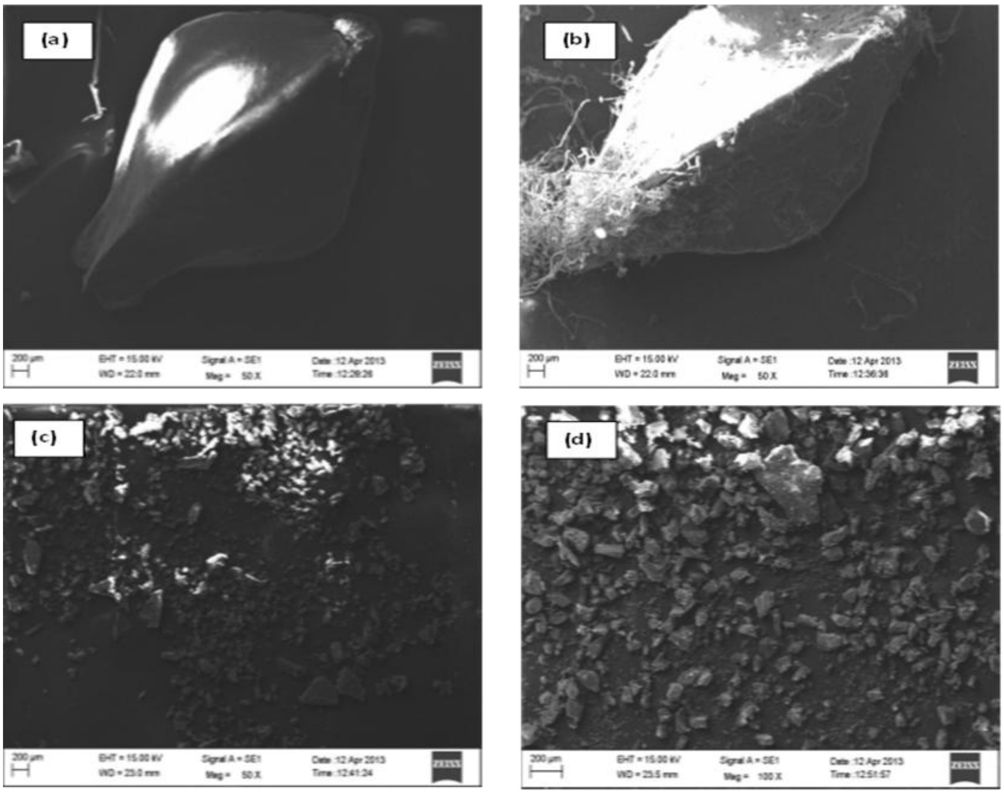


Fig 1: Scanning electron micrographs (a) UBW (b) GBW (c) UBF (d) GBF

3.2 Effect of germination on thermal properties of buckwheat flour

Thermal properties are used to understand the behaviour of starch containing foods. In current investigation the energy change in flour sample was measured at a fixed temperature range during heating and cooling in form of thermograms. The results obtained are described in terms of heat flow (mW), specific heat (C_p) and enthalpy change (ΔH). The heat flow was measured as the function of temperature (°C). The thermograms were recorded in convention endotherm in upward direction. It was observed as flour from ungerminated buckwheat grains attained T_o dissociation of starch started progressively at 36.57°C thus giving the curved line preceded to this point. The curved line signified heat intake was not linear with respect to increase in temperature. As temperature was increased curve changed to linear form and the relation became directly proportional till temperature reached 78.74°C as shown in Figure. 2 (a). The effect of temperature for GBF is shown in Figure. 2 (b) At this point complete dissociation of starch linkage *viz.*, 1-4 α-D glucopyranosyl took place and amylose oozed out of starch granule. At T_p the complete melting of starch in the flour took place which is glass transition temperature (crystalline to amorphous form). Finally the enthalpy reduced and association of bonding occurred according to the phenomenon as by Tester & Morrison (1990) [21]. Germination caused a significant increase in T_o while a significant decrease in T_p, T_e and ΔT (T_e - T_o) for flour derived from germinated and ungerminated buckwheat grains as shown in Table 1. It was observed ΔT for flour obtained from germinated grains (43.45°C) was lower than ungerminated grains (53.73°C) this might be due to presence of more amylopectin which gave crystallinity to starch present in flour derived from ungerminated buckwheat grains. The presence of amylose lowers the melting temperature of crystalline regions which resulted in lowering of gelatinization enthalpy for ungerminated buckwheat grain.

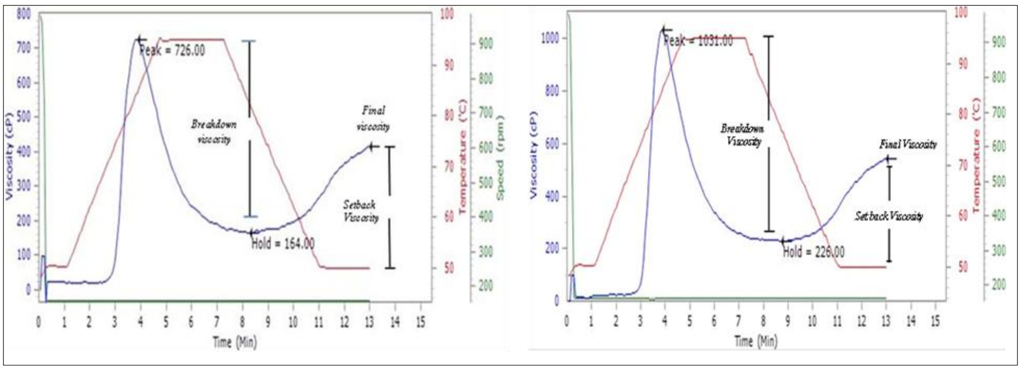
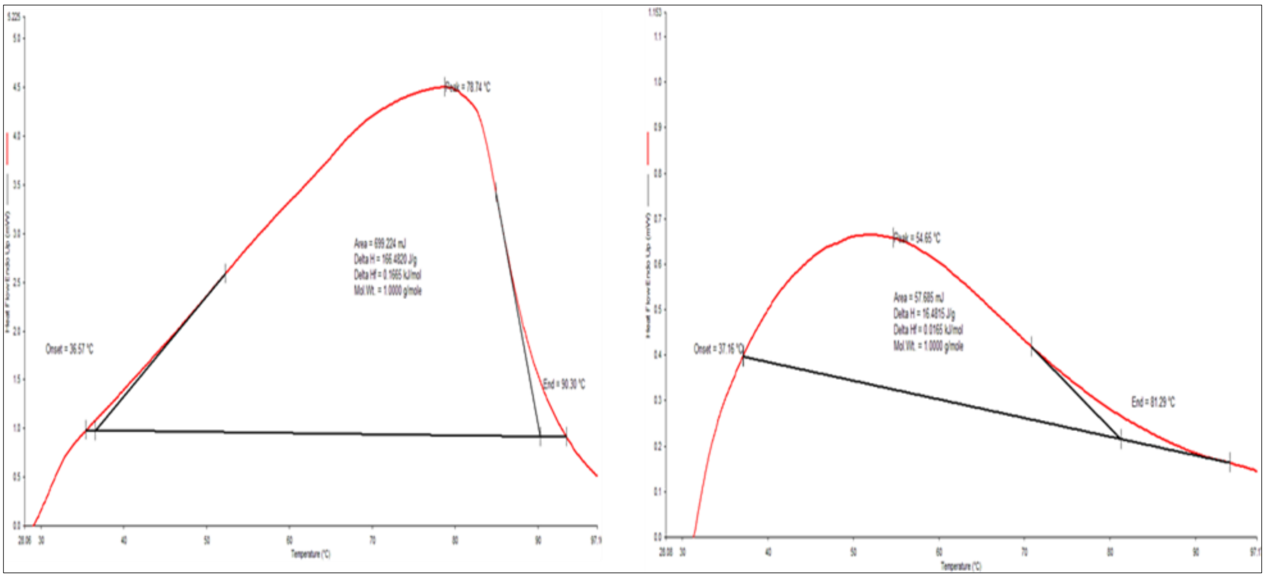


Fig 2: Pasting profile (a) UBF (b) GBF



(a) (b)

Fig 3: Differential scanning calorimetry (a) UGF (b) GBF

3.3 Effect of germination on pasting properties of buckwheat flour

Pasting temperature (PT) indicates minimum temperature at which swelling of starch granule starts when suspended in water. It depends upon rigidity and heating temperature of starch which affect leaching of amylose from starch molecule. It signifies the energy status of starch molecule when controlled heating and cooling is applied in cyclic manner. Energy status helps in selection of thickening and binding agents in food processing industries (Bao, 2008) [5] which helps in prediction of texture and final quality of food product. RVA under controlled temperature and fixed shear rate was used to determine the effect on viscosity of buckwheat flour as shown in Table 2. PT for flour obtained from ungerminated and germinated buckwheat grains was 74.30°C and 73.45°C respectively. A significant difference was observed between them. The higher PT for ungerminated flour indicated the presence of starch that is highly resistant to swelling and rupturing which required longer time for swelling. In flour derived from ungerminated buckwheat grain a decrease in number of free sites on glucose moiety of starch molecule has been observed which are responsible to form hydrogen bond, van deer wall interaction with hemi-acetyl oxygen and hydroxyl group with water molecule. The absence of such types of bonding results to harden of starch molecule, thus higher pasting temperature. Shevkani *et al*. (2015) [18] also reported similar results for amaranth flour which is also a good source of starch content. Hence, germination allowed cooking at lower temperature to get same degree of swelling**.**

As temperature increased the viscosity was found to be increased linearly. This might be due to linear increase of free sites available on starch molecule which gets bonded with water molecule. Thus it indicates the complete water holding capacity of grain or flour granule. The overall affect causes the increase in viscosity to maximum level called as Peak viscosity (PV) which expresses viscous load. Hence, PV is the point where the maximum swelling of starch granule takes place prior to sample cooling (Sanni *et al*., 2006; Adebowale *et al*., 2008a) [17, 1]. It gives an indication about thickening behaviour of a starch. In current investigation the peak viscosity for ungerminated and germinated flour was 1021±10 cP and 715.33±13.61 cP respectively. A significant decrease ($p<0.05$) in peak viscosity of the flour derived from germinated buckwheat grains was observed. This might be due to degradation of starch by enzyme during the germination process. It thereby which caused a significant increase of free sites available for water binding. Similar results have been reported by Osungbaro (1990) [14]; Sindhu & Khatkar (2016) [19] on 'Ogi' (A fermented Maize porridge) and buckwheat flour (*Fagopyrum Esculentum Moench*). The ungerminated buckwheat flour has high starch content (55%) than the one obtained from germinated grains. Thus the flour derived from ungerminated buckwheat grains has higher peak viscosity than the flour derived from germinated buckwheat grains.

Trough value (TV) of flour obtained from ungerminated and germinated grains was 225±4.5 cP and 163.66±3.51 cP respectively. It was observed that slurry prepared from flour of ungerminated grains had higher trough viscosity than from germinated grains flour. This may also be attributed to starch degradation which caused a decrease in viscosity value. Trough value is the maximum viscosity value at the constant temperature phase of the RVA parameters and measures the gel ability to withstand breakdown during cooling.

Breakdown viscosity (BV) is the resistance offered by swollen starch molecule in which amylopectin is responsible for disintegration when the gelatinized starch slurry is heated and shear rate is applied. In this investigation the slurry was subjected to higher temperature and constant shear stress. The combined action of these two effects caused disruption of hydrogen bonding and van deer wall interaction which further caused the leaching out of amylose from the starch molecules into the solution. The slurry got aligned in the direction of shearing with breaking of swollen starch granule due to shearing acting. This viscosity of sample after shearing is termed as breakdown viscosity or shear thinning. Breakdown viscosity for flour derived from ungerminated and germinated grain was 804±3.60 cP and 574.66±11.15 cP respectively which indicates that germination provides higher stability to starch granule during shearing (rotation per minute).

Final viscosity (FV) depicts the gelling behaviour of starch. The slurries of flour derived from ungerminated and germinated buckwheat grain were found to have final viscosity of 411.33±10.26 cP and 533.33±10.26 cP respectively. The viscosity here indicated the ability of flour to form a viscous paste after cooking and cooling. Thus it gives an idea about resistance of starch to shearing action (Adebowale *et al*., 2008b) [2]. This increase in viscosity due to cooling effect which caused the re-association of starch molecule is called as retrogradation or setback viscosity. Retrogradation is reordering of broken bonds to form new tetrahedral shape starch molecule in which water molecule is imbibed. It caused a reduction in kinetic energy and then re-association of starch molecules particularly amylose. This phase of the pasting curve is commonly referred to as the setback. It mainly affects the textural property of food. The similar results were obtained by Kaur & Singh (2005) [11] while studying the pasting properties of Taro (*Colocasia esculenta* L.) flour. The result indicated that germinated flour possibly had less starch retrogradation in comparison to flour obtained from ungerminated flour.

Peak time (PT) is a measure of the cooking time required for the full gelatinization of starch. Peak time for flour obtained from ungerminated and germinated grains was found to be 5.47±0.21 and 3.87±0.21 min respectively. The ungerminated buckwheat flour had higher peak time. The large variation in peak time associated to macro level modification that occurred in grain morphology during germination. The decrease in starch content of buckwheat flour was observed with germination and this decrease might be due to increase in amylase (an enzyme breaks amylose) activity. The hydrolysis of starch ultimately leads to decreased in peak time for flour obtained from germinated grain.

Table 1: DSC characteristics of UBF and GBF.

DSC Characteristics	UBF	GBF
Onset temperature (T_o) (°C)	36.43±0.26^a	37.38±0.26^b
Peak temperature (T_p) (°C)	77.04±0.78^a	54.38±0.68^b
End set temperature (T_e) (°C)	91.10±1.08^a	81.58±0.97^b
Gelatinized temperature ($\Delta T = T_e - T_o$) (°C)	53.87±0.89^a	43.45±0.80^b
Gelatinization enthalpy (ΔH) (J/g)	15.75±0.80^a	16.60±0.80^b

DSC- Differential Scanning Calorimetry.
UBF- Un-germinated Buckwheat Flour.
GBF- Germinated Buckwheat Flour.
Mean ± S. E. (n=3), ^{ab} are significantly different at ($p < 0.5$) with each other row wise.

Table 2: Pasting properties of UBF and GBF.

Properties	UBF	GBF
Pasting temperature (◦C)	74.65±0.31^a	73.41±0.12^b
Peak viscosity (cp)	1021±10^a	715.33± 13.61^b
Trough viscosity (cp)	225±4.58^a	163.66±3.51^b
Breakdown viscosity (cp)	804±3.60^a	574.66±11.15^b
Setback viscosity (cp)	345.67±4.16^a	244.66±5.5^b
Final viscosity (cp)	411.33±10.26^a	533.33±10.26^b
Peak time (min.)	5.47±0.21^a	3.87±0.21^b

UBF- Un-germinated Buckwheat Flour.
GBF- Germinated Buckwheat Flour.
Mean ± S. E. (n=3), ^{ab} are significantly different at ($p < 0.5$) with each other row wise.

4. Conclusion

The aim of the investigation was to clarify the effect of temperature on morphology and gelling properties of buck wheat grain and its flour. Thermograms obtained from DSC have clearly thrown light upon the nutritional changes that took place during germination in buckwheat grain. Germination decreased the amylose content in the buckwheat grain which affected the functional properties of starch contained in flour obtained by milling of buckwheat grains. Hydrolysis further decreased the peak time and peak viscosity of flour derived from buckwheat grains. Germination increased total heat (ΔH) content of grains which affected the textural properties and water holding characteristics of milled grain *i.e.,* buckwheat flour. These modifications make buckwheat to be used as thickener and water binding agent in product formulation to meet the requirement of product.

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