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Meenu Rani
Division of Food Science and Technology, Sher-e-Kashmir University of Agriculture Sciences and Technology, Jammu, Jammu and Kashmir, India

Julie D Bandral
Associate Professor, Division of Food Science and Technology, Sher-e-Kashmir University of Agriculture Sciences and Technology, Jammu, Jammu and Kashmir, India

Monika Sood
Assistant Professor, Division of Food Science and Technology, Sher-e-Kashmir University of Agriculture Sciences and Technology, Jammu, Jammu and Kashmir, India

Sushil Sharma
Professor and Head, Division of Agriculture Engineering, Sher-e-Kashmir University of Agriculture Sciences and Technology, Jammu, Jammu and Kashmir, India

Sachin Gupta
Associate Professor, Division of Plant Pathology, Sher-e-Kashmir University of Agriculture Sciences and Technology, Jammu, Jammu and Kashmir, India

Gurdev Chand
Head, Division of Plant Physiology, Sher-e-Kashmir University of Agriculture Sciences and Technology, Jammu, Jammu and Kashmir, India

Corresponding Author:
Meenu Rani
Division of Food Science and Technology, Sher-e-Kashmir University of Agriculture Sciences and Technology, Jammu, Jammu and Kashmir, India

Effect of germination on physico-chemical and antinutritional factors of oats flour

Meenu Rani, Julie D Bandral, Monika Sood, Sushil Sharma, Sachin Gupta and Gurdev Chand

Abstract
This study was proposed to determine the effect of germination on chemical and antinutritional characteristics of oat flour. Germination of oats grains was done at 25±2°C, kept for 72 hours further germinated seeds were dried at 55- 60 °C and milled to obtained flour. The germinated and non-germinated oats flour were comparatively evaluated for physico-chemical composition and antinutritional factors. Results obtained for protein and fiber content varied significantly was found to be 15.79%, 12.50% and 6.72%, 5.90% for germinated and non-germinated oats flour respectively. Physical properties of germinated and non-germinated oats flour showed L* value showed decreased trend while a* and b* value of oats flour increased after germination. Further the antinutritional properties indicated that tannin and phytic acid content showed the decreased trend after germination. Therefore, germination can improve the nutritional value and stability of oats flour.

Keywords: Antinutritional factors, biochemical changes, germination, nutritional value, oats, physcio-chemical properties

Introduction
Oats (*Avena sativa* L.) is a minor cereal grown as a multipurpose crop for grain, pasture and forage or as a rotation crop in many parts of the world. It is locally known as "*jai*"or "*javi*" and are grown in the Indian states of Himachal Pradesh, Punjab, Haryana, Uttar Pradesh and limited areas in Madhya Pradesh Orissa, Bihar, West Bengal (Ahmad *et al.*, 2014) [2]. The annual production of oat in India is about 12.10 tonnes per hectare (Anonymous, 2018-19) [5]. Now-a-days oats have received considerable attention for their excellent content of dietary fibres, phytochemical and high nutritional value. Oat grain have been recognized as functional foods, because, it possesses various health benefits such as hypo-cholesterolemic and anticancerous properties and also decrease the risk of various diseases. Recently they have also been considered helpful in the diet of celiac patients (Rasane *et al.*, 2015). Owing to their high nutritional value, oat-based food products like flakes, breads, cookies, probiotic drinks, biscuits, breakfast cereals and infant food are gaining more consideration. Oat consumption in human diet has increased because of health benefits associated with dietary fibres such as β-glucan, functional protein, lipid, starch components and phytochemicals present in the oat grain. Whole oat grain contains considerable amount of valuable nutrients such as dietary fibre as soluble and insoluble fractions, proteins, starch and unsaturated fatty acids. It also contains micronutrients such as vitamin E, folates, selenium, copper, manganese, carotenoids, betaine, choline zinc, iron, sulphur containing amino acids, alkyl resorcinols, lignins, lignane and phytic acid (Flander *et al.*, 2007) [12]. Oat products are well accepted in human nutrition. Compared with other grains, the oat contains high concentration of protein with beneficial amino acid composition, advantageous profile of fatty acids, with high amount of PUFA, excellent source of different dietary fiber, starch, phenolic compounds, minerals, vitamins and antioxidants (Butt *et al.*, 2008) [9]. Oat protein is nearly equivalent in quality to soya protein which has been shown by the WHO to be equal to meat, milk and egg protein. Major nutritional composition of oat have been reported as 13-20% protein, 2-12% crude fat (Aro *et al.*, 2007) [7], about 60% starch (Hoover *et al.*, 2003) [15] and 2.0-7.5% β-glucan (Annica and Desirée, 2011) [4]. Additionally, oats are source of several natural antioxidants such as tocopherols, alkylresorcinols, sphenolic acids and their derivatives, and a unique source of avenalumic acids (ethylenic homologues of cinnamic acids) and avenanthramides (N-cinnamyl anthranilate alkaloids), which are not present in other cereal grains (Mattila *et al.*, 2005) [29].

All of these phenolic compounds possess potential health-promoting properties because of their membrane-modulating and effects antioxidant activities.

The process of germination of cereal grains has been used for centuries for the purpose of softening the kernel, improving its nutritional value and reducing anti-nutritional factors. In fact, the germination method isused to improve the functionality of cereals like oat protein. (Kaukovirta-Norja *et al.*, 2004) [18]. During germination, oat seed proteins were degraded to increase the soluble protein content (Wu, 1983) [41] and the oat protein properties were improved without any chemical modifications being required. Germinated cereal grains also shows higher antioxidant activity and total phenolic content than those of un-germinated cereal grains and enhances their nutritional quality. During germination, intense desirable changes in the nutritional, biochemical and sensory characteristics of cereals occur due to degradation of reserve materials for respiration and synthesis of new cell constituents for developing embryo in the seed (Sharma *et al*., 2016) [34]. The benefits achieved are biological value of grains increases, the content of vitamins B_2, E and niacin, total sugar, dietary fibre and glucosamine increased, vitamin C is synthesized and the amount of irreplaceable amino acids is increased during hydrolysis of protein (Rakcejeva, 2006) [31]. The present study was undertaken to determine the effect of germination on physico-chemical and antinutritional properties of oats flour. The germination of a grain or seed is a chain of events that commences when viable, dry seeds absorbs water, and terminates with the elongation of the embryonic axis. Upon imbibitions, the quiescent seed rapidly resumes metabolic activity, including respiration, enzyme and organelle activity, and RNA and protein synthesis. Enzymes are synthesized to degrade storage macromolecules. These reactions lead to structural modification and development of new compounds, many of which have high bioactivity and can increase the nutritional value and stability of grains. Furthermore, many of the developed compounds are flavor precursors participating in the formation of palatable malt flavor (Warle *et al*., 2015).

Materials and Methods

Oats grains were procured from local market of Jammu and were analyzed for physico-chemical composition and antinutritional factors.

Preparation of sample

The oats were soaked in water twice their volume for 12 hour at room temperature (28-30 ^{0}C). After soaking, oats grains were evenly spreaded on germination sheets and covered with the same sheets. The grains were germinated at 25±2 ^{0}C germinated for 72 hour at 40-45 ^{0}C in a BOD Incubator (Super-Deluxe York Scientific Industry). During this period grains were kept moist by sprinkling water twice daily. The germinated grains were washed and dried in hot air oven at 60 ^{0}C till the moisture reached to 10%. The dried grains were milled into flour using flour mill (using grinder, Model) and the resultant flour was sieved trough 80-100 mesh sieve, packed in aluminum laminates and stored at room temperature for further use.

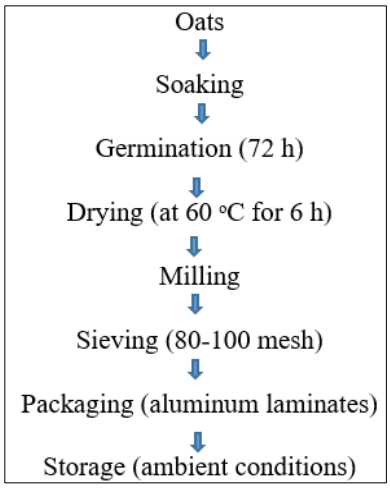

Fig 1: Flow chart for preparation of oats flour

Physical properties

Colour analysis (L*, a*, b*)

The colour of sample was measured using a Hunter's lab colour analyzer (S.No.CX2013). The equipment was calibrated using white and black standard ceramic tiles (Younis and Ahmad, 2015) [42].

Water activity

The water activity (a_w) of samples was determined at 25℃ using an Aqualab Series 4TE water activity meter (Decagon, Pullman, WA, USA) Abdullah *et al.* (2018).

Chemical parameters

Moisture Content (AOAC, 2007) [6]

Moisture content was determined adopting AOAC (2007) [6] method as following:

$$\text{Moisture (\%)} = \frac{\text{Loss in weight (g)}}{\text{Weight of sample (g)}} \text{x } 100$$

Crude protein

The crude protein content was determined by micro Kjeldahl method, using the factor 6.25 for converting nitrogen content into crude protein (Sadasivam and Manickam, 2008) [33]. Protein content was calculated using the equation below.

$$\text{\% Nitrogen} = \frac{\text{Titre value} \times 0.00014 \times \text{Volume made}}{\text{Aliquot taken(g)} \times \text{Weight of sample (g)}} \times 100$$

% crude protein =% Nitrogen × 6.25

Crude fat

Crude fat was determined by the Soxhlet extraction technique (AOAC, 2007) [6]. Fat content was calculated by the following equation.

$$\text{\% crude fat} = \frac{\text{Amount of evaporated residue (g)}}{\text{Weight of sample (g)}} \times 100$$

Crude fibre

Crude fibre was determined by using ztandard method (AOAC, 2007) [6] and was calculated as follows.

$$\% \text{ crude fibre} = \frac{\text{Loss in weight on ignition (g)}}{\text{Weight of sample (g)}} \times 100$$

Total Ash

Ash content of the sample was determined by method as described in AOAC (2007) [6]. Ash content was calculated by using following equation:

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

Total Carbohydrate

The carbohydrate content was estimated by the difference method. It was calculated by subtracting the sum of percentage of moisture, fat, protein and ash contents from 100 according to AOAC (2007) [6].

Total carbohydrate (%) = 100-(moisture%+crude fat%+crude protein%+total ash %)

Total energy

The total energy value of the food formulation was calculated according to the method of Mahgoub (1999) using the formula as shown in the following equation:

Total energy (K cal/100g) = [(% available carbohydrates × 4) + (% crudeprotein × 4) + (% crudefat × 9)]

Anti nutritional factors

The tannins were determined by known method Makkar *et al.* (1993) [28] with some modifications. The tannins content were calculated from calibrated curve, using tannic acid as standard. The results were expressed as mg TAE/100g. Phytic acid content was estimated by known method of Sadasivam and Manickam (2008) [33].

Results and Discussions

Physical properties of oats grain various physical properties of oats grain were determined, and results obtained are presented in Table 1.

Table 1: Physical Parameters of oat grain Physical

Physical properties	Non-germinated oats flour	Germinated oat flour
L* (Lightness)	52.80±2.51	33.26 +0.03
a* (Redness or greenness)	1.86±0.06	2.01 +0.02
b* (blue or yellowness)	6.62±0.05	6.80 +0.01
Water activity (a_w)	0.70±0.04	0.40 +0.05
Moisture (%)	9.52±0.02	3.97 +0.04

The data given in Table 1 revealed Various Physical characteristics of oats flour. Colour is an important characteristic for determining the visual acceptance. Results revealed that among physical parameters, L* colour value of oats flour decreased after germination while as a* and b* value of oat flour increased. The L* colour value was reduced from 52.80 to 33.26 whereas a* and b* values were increased from 1,86 to 2.01 and from 6.62 to 6.80 after germination. Table1 further summarize that the moisture content and water activity of raw oats flour was found to be 9.52% and 0.70 while after germination followed by drying it was found to be 3.95% and 0.40 respectively. Liu *et al.* (2018) [25] and Slathia *et al.* (2016) [35] also reported decrease in L* value and increase in a* and b* value of germinated mungbean flour. The decreased L* value could be due to the secondary reactions capable of causing browning during and after cooking (Akissoé *et al.*, 2005) [3]. As per Sofi *et al.* (2020) [37] the L* value of chickpea flour decreased with the increase in germination time, while b* and a* value increased with the increase in the germination time of chickpeas. According to Li *et al.* (2020) [24] germination process activates various enzymes such as amylase and protease that cause a significant increase in sugars, peptides, amino acids and non-starch polysaccharide which may compete between starch and these materials for available water and a consequent reduction in water activity (Chung *et al.*, 2012) [11]. Similar results were reported by Sofi (2018) [36] in germinated chick pea flour and Gernah *et al.* (2011) [13] in malted maize flour. Decrease in the moisture content of wheat and barley grain after sprouting and roasting were also reported by Kour (2014) [22] and the finding is in good agreement with our results.

Table 2: Proximate composition of oats flour

Chemical properties	Mean value*	
	Non-germinated oats flour	Germinated oat flour
Crude Protein (%)	12.50±0.33	15.79+0.03
Total ash (%)	6.23±0.04	2.4+0.02
Crude Fat (%)s	5.90±0.05	5.76+0.01
Crude Fibre (%)	2.15±0.01	6.72+0.04
Total carbohydrate (%)	69.43±0.42	68.61+0.05
Total energy (Kcal/100g)	379.00±5.12	360.00 +0.24

*Each value represents the average of three determinations

Results given in above Table. 2 indicated that the moisture content of non-germinated and germinated oats flour varied between 11.2 to 12.51% it means moisture content was increased after germination similar to the results were reported by Khatoon, and Prakash, (2006) [21]. The total protein increases after germination from 12.50 to 15.79%. Similar result also reported by Khader, (1983) [20]. Crude fibre were intensively increased with the germination of barley from 2-15 to 6.72%. Table. 2 further showed that carbohydrates content, ash and crude fat in barley before and after germination ranged from 69.42 to 68.61, 6.23 to 4.60 and 5.90 to 5.76% respectively. Moreover, Chauhan *et al.* (2015) [10] showed that there was significant difference in carbohydrate content among amaranth before and after germinated flours. The decrease in carbohydrates in germinated grains may be attributed to increase in alph-amylase activity which breakdown complex carbohydrates into simpler and more absorbable sugars was reported by Hung *et al.* (2012) [16]. Karuma *et al.* (2018) [17] reported significant changes in biochemical and nutritional characteristics of oat and barley with germination which results in high protein content of germinated oats and barley when compared to ungerminated oats and barley grains. Increase in protein content during germination might be due to biological synthesis of new amino acids was also reported by Sofi (2018) [36] and Uppal and Bains (2012) [39] in chickpea flour. Beniwal *et al.* (2019) [8] studied the effect of pretreatment methods *viz.*, cooking, roasting, germination on quality of amaranth and quinoa flour and reported decrease in crude fat content thus supporting our findings. Kavitha and Parimalavalli (2014) [19] reported decrease in fat content of germinated cereal and legume flour. The increase in crude fibre content during germination might be due to intense biochemical processes that activate the grain and resulted in

increased fibre content of cereal grains (Rakcejeva, 2006) [31]. Similar trend of increase in crude fibre content was reported by Gernah *et al.* (2011) [13] in germinated maize flour and Lemmens *et al.* (2019) [23] in sprouted grains. The results are in accordance with those reported by Tian *et al.* (2020) [38] who reported that the reduction in carbohydrate content during germination was due to the activity of α-amylase enzymes. As per Kour (2014) [22] carbohydrates are used as a energy source during germination process for respiration and metabolic activities during sprouting and malting. Liu *et al.* (2018) [25] also reported that soaking and germination of maize grain for 48 and 72 h resulted in the reduction of carbohydrate content when compared with raw samples thus confirmed our results.

Table 3: Antinutritional factors of oats flour

Antinutritional factors	Non-germinated oats flour	Germinated oat flour
Tannis (mg TAE/100g)	42.60±0.55	27.99+0.06
Phytic acid (mg/100g)	1818.75±56.00	1630.59+0.45

Results given in above Table 3 indicated that the antinutritional composition *viz*., The tannin content and phytic acid content corresponding to the values of were recorded in raw oat flour before and after germination were ranged from 42.60 to 27.99 TAE per 100g and 1818.75 to 1630.59 mg per 100g. Results reported are in close agreement with the findings of Pal *et al*. (2016) [30], Handa *et al.* (2017) [14] and Luo *et al.* (2020) [26].

Conclusion

In the present study germination of oats was carried out under the controlled conditions of soaking, germination and drying. From the result, it can have concluded that the physico-chemical and antinutritional properties were significantly influenced by the germination process. A significant decrease crude fat, ash and carbohydrate of oats were estimated. Moreover the antinutritioal factors were reduced by the germination process. Further the nutritional properties indicated that antioxidant activity and total flavonoids shown to increase for germinated flour. Therefore, germination can improve the nutritional value and stability of oats flour.

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