Physical characteristics and effect of germination on functional properties of black soybean (*Glycine max*)

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**ABSTRACT**

The present research analyzed the physical and functional properties of black soybean. Germinated sample was prepared by soaking grains for overnight and allowed for germination for 72-hours in incubator at 32°C, dried, milled and kept in air tight containers for further analysis. The results showed that black soybean seeds had good physical properties. Functional properties showed that germinated seed flour had significantly higher water absorption capacity (203.33ml/100g) and foaming capacity (22.67%), while raw black soybean seeds flour had higher emulsion activity (49.46%), emulsion stability (47.67%), fat absorption capacity (126.67ml/100g) and foam stability (95.35%). Both the samples had the highest solubility at alkaline medium. The results of particle size distribution showed that both the flour samples had maximum retention on 85 mesh sieve. The study concluded that black soybean has good physical and functional properties which makesit potentially ideal for local food uses and industrial food systems.

**Key words:** Black soybean, Germination, Functional characteristics, *Glycine max*, Physical properties.

**INTRODUCTION**

Soybean (*Glycine max* (L.) Merril), known as *Garikala* in West Bengal and *Muth* in Kashmir is a member of Papilionaceae family, native to North-eastern China and distributed in Asia. It is an important legume which contains 37–42% protein, 35% carbohydrates and 17–24% oil (USDA, 2009) that serves as an excellent source of oil and protein for human consumption and animal feed. Soybean is also known as “Meat of the field” in the Orient and “Cindrella crop” in the USA (Ghosh and Jayas, 2010). The black varieties of soybean traditionally grown on a small scale in Himachal Pradesh, Kumaon hills of Uttarakhand, Eastern Bengal, Khasi hills, is believed to be introduced via Burma by traders from Indonesia and has occupied important place in staple diet. In Uttarakhand region of India, these soybean varieties are known as *Bhatt* (Barh et al., 2014). *Bhatt*, in Uttarakhand, is grown over 6799ha area with the productivity of 6797 t and 9.99q/ha respectively (Agriculture Statistics data on *kharif* crops, 2015-16). Consumption of black soybean is rapidly growing due to their nutritional values and potentials to develop as healthy functional food ingredients in various parts of the world.

Quality of a food product is characterized by its structure, nutritional value and/or acceptability. Legumes are widely used in the food industries for their many functional properties (Chef-Guerrero et al., 2011). Physical properties of food grains play an important role in understanding their cooking and processing properties. Functional properties give information on how foods behave in a system either as a processing aid or as a direct contributor of product attributes (Oyebode et al., 2007).

Research work related to physico-chemical properties has been reported on the yellow and white varieties of soybean but scanty information is available for black soybean. Therefore, the aim of this study was to investigate the physical and functional properties of black soybean along with the assessment of impact of germination on these properties.

**MATERIALS AND METHODS**

The present research was conducted in the months of March and April, 2016. Black soybean (local variety) was procured from Tarai Development Corporation, Haldi, Distt. U.S. Nagar and brought to laboratory in poly bags for investigation. For estimation of physical characteristics, whole grains were used. For functional properties, flour was prepared using germination treatment. For preparation of germinated sample, black soybean grains were cleaned to remove dust, grit and other impurities, followed by washing of grains in clean water, overnight soaking of grains and draining. Next day, soaked grains were kept for germination for next 72 hours at 32°C in incubator. Germinated grains were oven-dried at 65°C for 6 hours, followed by grinding into flour, screening through 60 mesh sieve and storing the flour in a dry and air tight container for further analysis. Raw black soybean flour (not germinated) was used as control.

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Physical characteristics of seeds: Physical characteristics viz seed weight, seed volume, seed density, hydration capacity, hydration index, swelling capacity and swelling index were analyzed using the methods given by Williams et al. (1983) and bulk density of seeds was estimated by the method given by Asoegwu et al. (2006).

Functional properties of flours: Water and fat absorption capacity of flours were measured by the centrifugation methods given by Smith and Circle (1972) and Lin et al. (1974), respectively. Emulsion activity and stability were determined using the method of Yatsumatsu et al. (1972). Foam capacity and foam stability were measured according to the method reported by Lawhon et al. (1972). Protein solubility was assessed by the method given by Ma (1983) using micro-kjeldahl method. Particle size distribution was determined according to the method given by Bedolla and Rooney (1984).

Statistical analysis: Experiments were carried out in triplicates. All the quantitative data was computed in terms of mean and standard deviation. Data was subjected to one-way analysis of variance (ANOVA) to determine significant difference between two means at 5% level of significance.

RESULTS AND DISCUSSION

Physical characteristics of black soybean seeds: Physical properties of seeds are important for the design of equipment necessary for harvesting and post-harvest handling, transportation and processing of agricultural produce into different consumable and marketable food items. Various types of unit operations such as cleaning, grinding and sorting are designed on the basis of the physical properties (Kumari and Raghuvanshi, 2015). As evident from Table 1, one hundred seed weight and volume of black soybean was 8.55g and 8.33ml, respectively. The mean 100 seed weight of black soybean in present study was lower as compared to the value reported by Acuna et al. (2012). Seed volume is important from handling point of view as higher seed volume affects transport and handling. A 100 seed volume of 8.33ml was found parallel to the finding of Sharma et al. (2014) who observed volume of soybean seeds ranging from 8.1 to 12ml in 10 different genotypes of soybean.

Bulk density of black soybean seeds in the present study was found to be 0.61g/ml which is lower than 0.97g/ml reported by Acuna et al. (2012) but higher than the observation of Singh (2017) as 0.33g/ml. The factor affecting bulk density of grain is starch polymer structure. The loose polymer structure results in low bulk density (Iwe and Onadipe, 2001). Hydration capacity of the grains is an important attribute which affects the cooking quality and in turn, organoleptic qualities of the product. The variation found is due to differences in bran layer as it hinders the absorption of water (Malleshi and Desikachar, 1985). Hydration capacity of seeds in the present study was 11.69g/100 seeds which was found lower than the value reported by Acuna et al. (2012) as 20g/100 seeds but in accordance to the values quoted by Sharma et al. (2014) ranging between 8 to 12 g/100 seeds.

Swelling capacity gives an indication of increase in the volume upon absorption of water. It is a very important parameter as changes in volume during processing may change the acceptability of the final product (Ayodele and Beatrice, 2015). Swelling capacity, hydration index and swelling index of 100 black soybean seeds in the present study was found to be 29ml, 1.23 and 1.03 respectively. Higher hydration capacity and swelling capacity permits the grain to absorb more amount of water thereby rendering the grains soft.

Functional properties of flours: Functional properties are the fundamental physicochemical properties that reflect the complex interaction between the composition, structure, molecular conformation and physico-chemical properties of food components together with the nature of environment in which these are associated and measured (Siddiq et al., 2009). The data on water and fat absorption capacity of raw and germinated black soybean seeds flour is given in Fig. 1.

Water absorption capacity is a measure of trapped water that includes both bound and hydrodynamic water. It affects the texture, juiciness and taste of the products and plays an important role in the food preparation process because it influences other functional and sensory properties.

| Table 1: Physical characteristics of black soybean grains |
|----------------------------------------------------------|
| **Physical properties**                                  | **Black soybean** |
| Seed weight (g/100 seeds)                               | 8.55±0.22         |
| Seed volume (ml/100 seeds)                              | 8.33±0.58         |
| Seed density (g/ml)                                     | 1.03±0.04         |
| Hydration capacity (g/100 seeds)                        | 11.69±0.38        |
| Hydration index                                         | 1.23±0.02         |
| Swelling capacity (ml/100 seeds)                        | 29.00±0.0          |
| Swelling index                                          | 1.03±0.02         |

Fig 1: Water and oil absorption capacity of raw and germinated black soybean flours
Water absorption capacity of black soybean flour in the present study increased from 197 to 203.33ml/100g on germination, although it was non-significant (p≤0.05). Elkhalifa and Bernhardt (2010) and Ghavidel and Prakash (2006) reported the same effect of germination on water absorption capacity of cowpea and sorghum flours, respectively. An increase of WAC on germination could be attributed to an increase in protein content and change in the quality of protein upon germination and also breakdown of polysaccharide molecules, hence the sites for interaction with water and holding water would be increased.

Sharma et al. (2014) observed water absorption capacity of 10 different genotypes of golden soybean flours within a range of 94.3 to 119.50ml/100g. Agume et al. (2017) and Chen et al. (2017) reported higher values for WAC of soybean flour as 313 and 427ml/100g, respectively. Value of water absorption capacity of the present study falls in between the values quoted by Sharma et al. (2014) and Chen et al. (2017), which may be due to the varietal variation and place of cultivation of soybean. Singh (2017) reported WAC of 257 and 307ml/100g for the raw and malted black soybean whereas Marquezi et al. (2017) observed WAC of 130.2 and 137.4 ml/100g for the black varieties of common bean.

Fat absorption capacity of food is attributed to the physical entrapment of oil which is considered important as a flavour retainer and improves mouth feel of foods. Flours from legume samples that had FAC of more than 6.0% have been reported to perform well in formulation of meat extenders and bakery products (Yadahally et al., 2008).

Fat absorption capacity of germinated black soybean flour (93.3ml/100g) was significantly lower than its raw counterpart (126.6ml/100g). The decrease in fat absorption capacity might be due to the negative effect of germination on the hydrophobicity of legume proteins (El-Adawy et al., 2003). Various studies have reported higher values for fat absorption capacity in different pulses (Acuna et al., 2012, Agume et al., 2017).

Foams are gaseous droplets encapsulated by a liquid film containing soluble surfactant protein resulting in reduced interfacial tension between gas and water (Kaushal et al., 2012). Germination led to significant (p≤0.05) increase of 35.29% in foaming capacity (Table 2). This may be attributed to increase in total soluble protein of germinated seed flour. Foaming capacity of 14.67% in present study is lower than the values reported by Oshodi and Ekperigin (1989) and Acuna et al. (2012) as 68% in pigeon pea flour and 37.21% in soybean flour. Chen et al. (2017) reported lower values for foaming capacity of black soybean as 12.5%. Foaming properties are the functional properties, where aeration and overrun are required e.g. whipped toppings, baked foods and ice-cream mixes. Hence, black soybean flour might be used as a potent foaming agent in food industry.

Foam stability is important because the usefulness of whipping agents depends on their ability to maintain the whip as long as possible (Lin et al., 1974). Raw black soybean flour in present study had foam stability of 95% which is higher than the values reported by Oshodi and Ekperigin (1989) and Acuna et al. (2012). Foam stability was significantly (p≤0.05) decreased in germinated seed flour. This finding is in accordance to the observation of Ghavidel and Prakash (2006) who reported increase in foaming capacity and decrease in foam stability in germinated cowpea, lentil, green gram and bengal gram.

Emulsion activity and stability of raw and germinated black soybean flour (49.46% and 47.67%) was found significantly higher (p≤0.05) than germinated (42.13% and 41.71%) black soybean flour. The finding of this study with respect to emulsion activity and stability of raw black soybean flour was found parallel to the values reported by Oshodi and Ekperigin (1989), but lower than the findings of Chau and Cheung (1998). Acuna et al. (2012) reported lower values for emulsion activity and stability.

The differences among the emulsion activities and emulsion stabilities are related to the protein contents (soluble and insoluble) and other components, such as astarch, fat, and sterol contents, of the legume flours. The capacity of proteins to enhance the formation and stabilization of emulsion is important for many applications in cakes, coffee whiteners, and frozen desserts. In these products varying emulsifying and stabilizing capacities are required because of different compositions and stresses to which these products are subjected (Elkhalifa and Bernhardt, 2010).

Amongst all the functional properties of legumes, protein solubility is probably the most critical and complex property because it affects other properties such as emulsification, foaming and gelation (Kinsella et al., 1985). The protein solubility profiles of processed black soybean flour shown in Fig. 2 revealed that both the samples had highest solubility at alkaline medium. Minimum protein solubility for both the samples occurred between the pH 4 and 5, which is the isoelectric pH. The protein solubility increased at pH values on each side of the pI region. These findings agree with the results of Oyebode et al. (2007) for Adenopus benth seed. Similar findings have been reported by Oshodi and Adeladun (1993) on Lima bean and Ayodele and Beatrice (2015) on various underutilized legumes of Nigeria.

### Table 2: Functional properties of raw and germinated black soybean flour

| Functional properties | Raw | Germinated | CD at 5% |
|-----------------------|-----|------------|----------|
| Foaming capacity (%)  | 14.67±1.15 | 22.67±5.03 | 0.37     |
| Foam stability (%)    | 95.35±0.99 | 87.59±2.08 | 1.34     |
| Emulsion activity (%) | 49.46±1.64 | 42.13±1.03 | 1.04     |
| Emulsion stability (%)| 47.67±1.70 | 41.71±1.77 | 1.38     |
Table 3: Particle size distribution of raw and germinated black soybean flour

| Particle size      | Raw          | Germinated |
|-------------------|--------------|------------|
| 16 mesh sieve (%) | 0.18±0.01    | 0.17±0.01  |
| 36 mesh sieve (%) | 20.84±0.29   | 17.57±0.11 |
| 60 mesh sieve (%) | 23.76±0.31   | 19.33±0.28 |
| 85 mesh sieve (%) | 48.47±0.33   | 56.77±0.31 |
| 100 mesh sieve (%)| 6.48±0.58    | 5.61±0.46  |
| Base              | 0.35±0.03    | 0.56±0.04  |

Results of particle size distribution showed that both the flour samples had maximum retention on 85 mesh sieve with 48.47% in raw and 56.77% in germinated black soybean flour (Table 3) and minimum retention on 16 mesh sieves. The particle size has influence on chemical and physical properties of the flour. The shape and size distribution of particles can have an important impact on many aspects of food including taste, texture, appearance, stability, processability and functionality of the final product (Sakhare et al., 2014).

CONCLUSION

The knowledge of various physical and functional properties of legumes provides the data required for evaluating and retaining the quality of final products and also gives indication of how they would behave in a food system. Like the other varieties of soybean, black soybean was found to be having good functional properties such as swelling, foaming and emulsion capacities along with protein solubility, which may improve textural and quality characteristics of the products. Although the black soybean was found more soluble in the alkaline medium, it can be utilised in the formulation of both acid and alkaline foods.

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