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Characterization of Functional Flour from Germinated Grains

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ABSTRACT

More than 1% world's population is suffering from celiac disease arising from gluten consumption and malnutrition arising from lack protein and micronutrients consumption. Germination of grains has been reported improving nutrient bioavailability, protein digestibility and sensory properties. The present study aimed to characterize flours obtained from these germinated grains viz., buckwheat, finger millet and paheli dal. The signigicant level moisture content (11.21 to 12.10%), Fat (2.13 to 2.6%), ash (2 to 3.83%), fiber (3.5 to 5.80%), protein (8.20 to 23.43%), and carbohydrates (53.63 to 69.17%) was found in germinated grain's flour. However, functional properties were bulk density (0.75 to 0.83 g/mL), dispersibility (51 to 76%), water absorption capacity (1.47 to 2.4 mL/g), oil absorption capacity (1.73 to 2 mL/g), emulsifying activity (0.489 to 0.503%), foam capacity (108 to 115%), swelling index (1.7 to 2.73 g/g) and swelling capacity (3.20 to 6.43 g/g).

Keywords: Buckwheat; Finger millet; Functional properties; Germination; Pasta.

INTRODUCTION

Food not only satisfies hunger and provides essential nutrients to humans but also prevents nutritional disorders and improves the physical and mental well-being of consumers (Menrad, 2003).

About 1% world's population is suffering from celiac disease due to gluten based diet (Plugis & Khosla, 2015). Currently, a life-long adherence to a gluten-free diet is the only treatment for the disease (O'Shea et al., 2014). Thus, it is an opportunity to use gluten free ingredients. Among the gluten free ingredients, millets (in particular, pseudo cereals) are having low glycemic index and also an excellent source of vitamins and minerals including fat soluble vitamin E which is an essential antioxidant.

Buckwheat, *Fagopyrum esculentum* Moench (Family: Polygonaceae), is a one of the underutilized gluten free pseudo-cereal, but known for its proteins, dietary fibre, catechins, polyphenols, rutin, minerals (micro elements *viz*. Zn, Cu, Mn, Se and macro elements *viz*., K, Na, Ca, Mg and vitamins (Steadman et al., 2000; Li & Zhang, 2001; Wei et al., 2003; Kim et al., 2004; & Stibilj et al., 2004).

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In addition, its protein has a high biological value because of its balanced amino acid composition (Pomeranz & Robbins, 1972). Finger millet or ragi (Eleusine coracana L.) is another underutilized millet primarily cultivated in different regions of India, and also Nepal, Sri Lanka, East China and Bangladesh, Kenya, Tanzania, etc (Hilu & DeWet, 1976). It has the highest calcium content amongst all cereals i.e. 344 mg/100 g. It contains 44.7% essential amino acids (Mbithi-Mwikya et al., 2000) of the total amino acids, which is higher than the 33.9% essential amino acids in FAO reference protein (FAO, 1991). Besides the nutrients, the flour obtained from the millet is gluten free and hence recommended for the patients suffering from celiac disease and also helps in the management of diabetes mellitus, obesity, hyperlipidemia, etc. Another important grain consumed in the Himalayan region is the paheli dal (black gram, Vigna Mungo. L) which is a source of protein for local tribes. It is one of the less studied legumes. Considering significance of the grains, the combination of cereal and legume proteins would provide a better overall balance of essential amino acids (Livingstone et al., 1993).

In addition, germination of grains have been reported to enhances the enzymatic activity of sprouting seeds, leading to the breakdown of proteins, carbohydrates and lipids into simpler form (Nout & Ngoddy, 1997). This processing method activates proteases which are active in degrading proteins, thereby increasing nutrient bioavailability (Taylor et al., 1985). That is, Germination increases protein digestibility and improves sensory properties (Eyzaguirre et al., 2006), and reduces the levels of anti-nutrients as well (Chavan et al., 1989).

Considering the significance of grain germination, the study was conducted to develop gluten free flours from selected germinated grains and their functional properties were determined to utilize them to produce composite flour and its subsequent use for product development.

MATERIALS AND METHODS

1.1. Materials

The raw materials, *viz.* finger millet, buckwheat and paheli dal were purchased from a local market (Lal Bazaar, Gangtok) of East Sikkim. The grains were cleaned and kept in metal bins at room temperature (25°C) till further use. Other important materials for characterization of functional flour such as chemicals, oil, muslin cloth, etc. were purchased from authentic source for the standard quality.

1.2. Flour preparation

Flour was processed as per standard procedures given in Figure 1. However the care was taken to minimize heat generation during grinding of germinated grains. A clean seeds were washed and steeped in the distilled water (1:2 w/v) at room temperature. Soaked seeds were strained off, rinsed with water and put into the damp muslin cloth in a tray. In order to maintain an adequate hydration level, grains were sprayed at regular interval with distilled water. Thereafter, the sprouted grains were dried at 55°C for 8-10h using a cabinet tray dryer. The moisture content of dried sprouts was 12% (w.b.). Dry grains were dehulled (except finger millet) using rice dehuller and after grounded in a domestic flour mill and sieved 300 µm to get fine flours.

1.3. Chemical analysis

The protein (Kjeldahl method, N×6.25), fat (Solvent extraction), fiber, ash and moisture were determined according to the AOAC (2000) methods. The digestible carbohydrates were calculated by difference, i.e. 100 - %moisture + % protein + % fat + % ash + % fiber. Analyses were performed in triplicates.

1.4. Functional properties *Bulk density*

Bulk density (BD) was determined by the method of Wang and Kinsella (1976) and calculated using formula:

Bulk density $(g/mL) = \frac{\text{Weight of sample}(g)}{\text{Volume of sample}(mL)}$

Dispersibility of flour was determined by method given by Kulkarni et al. (1991) and calculated by using formula: <i>Dispersibility</i> = 100 - volume of settled particles	determined by the method of Sosulski et al. (1976). Water and oil absorption capacity was expressed as gram of water and oil bound per gram flour as given in following expressions			
Water and oil absorption capacity	gram nour as given in following expressions.			
Water absorption capacity = $\frac{\text{volu}}{1}$	ume of water absorbed (mL) weight of the sample (g)			
For oil,				
Oil absorption capacity = $\frac{\text{volu}}{\text{w}}$	ume of fat absorbed (mL) reight of the sample (g)			
<i>Emulsifying activity</i> The emulsion activity was determined by the method of Yasumatsu et al. (1972). The	emulsifying activity was calculated using following equation.			
Emulsion activity = $\frac{\text{height of emulsion layer}}{\text{height of whole layer}} \times 100$				
<i>Foam capacity (FC)</i> The foaming capacity (%) of flours was measured the method described by Nath and	Rao (1981). The volume of foam after whipping for 30 s was expressed as foaming capacity.			
FC (%) = $\frac{\text{Volume of foam (AW) -Volume of foam (BW)}}{\text{Volume of foam (BW)}} \times 100$				
Where, AW: After whipping, BW: Before whipping				
<i>Swelling index</i> The method described by Ukpabi and Ndimele (1990) was used to determine swelling index	(SI) of the samples. It was calculated using following equation.			
$SI = \frac{Volume after soaking-Volume before soaking}{Weight of sample}$				
Swelling capacity	The gel obtained from swelling index was used in calculating swelling capacity (SC) (Ukpabi & Ndimele, 1990).			
Weight of col				

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$$\%SC = \frac{\text{Weight of gel}}{\text{Weight of sample}} \times 100$$

1.5. Statistical analysis

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Dispersibility

All the experiments were performed in triplicate and the average values are reported along with standard deviation. The data

obtained from the study were analyzed using analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) to determine the

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Water and Oil absorption capacity was

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significant difference between means using MS Excel-2010.

RESULTS AND DISCUSSION

1.6. Proximate composition of germinated grain flours

The data obtained for proximate composition of germinated buckwheat flour (GBF), finger millet flour (GFMF) and paheli dal flour (GPDF) is presented in Table 1.

The moisture content (w.b.) of GBF, GFMF and GPDF were in the range of 11.21 to 12.10%. From the present results, it was observed that GBF had highest moisture content 12.10% followed by GPDF 11.50% and GFMF 11.21%. As per Baljeet et al. (2010), moisture content of buckwheat flour was 11.60%; according to Wani et al. (2013) and Kavitha et al. (2013) the black gram flour had moisture content of 9.6-11.6%.

There was significant difference between protein content of GBF, GFMF, and GPDF. Among various GPDF showed the highest amount of protein about (23.43 %) and GFMF had lowest amount of protein content (8.20%). The studies showed that the protein content of buckwheat flour was 12.3 % (Li & Zhang, 2001), of finger millet flour was 8% (Ponnuthurai, 1989), and that of black gram seeds and flours was 21% (Kantha et al., 1987) and 26.75% (Girish et al., 2012), respectively. Several other researchers were also reported a wide variation in the protein content the depending on variety, but also environmental effects.

The ash represents the inorganic matter present in a particular sample (Table 1). Among the all, GPDF had contained highest amounts of ash content (3.83%) followed by GBF (2.11%) and GFMF (2%). The ash content of different varieties of black gram was varied between 2.7 to 3.3% as reported by Wani et al. (2013). An almost similar trend has been reported by Li and Zhang (2001) for buckwheat 2.11%. The ash content of finger millet was varied between 2.2-2.5% (Gull et al., 2015; Ravindran, 1991 & Ponnuthurai, 1989). The finding showed that the mineral content of paheli dal is comparatively higher.

Table 1 show that the fat content of GBF, GFMF and GPDF was 2.6, 2.5 and 2.13 %, respectively. The reported values are in conformity with Wang et al. (1995) and Li and Zhang (2001) who also reported that the fat content in buckwheat was 2.30%. Kavitha et al. (2013) reported that the fat content in different varieties of black gram ranged from 4.4 to 5.6 %. Whereas, Ravindran (1991) and Gull et al. (2015) reported that the value of ash content of finger millet ranged between 1.6 to 2.73%. The variation in the values of fat might be due to varietal difference or agro climatic conditions in which the crops were grown.

The data presented in Table 1 showed that the fiber content in GBF, GFMF, and GPDF were 5.80, 3.5, and 5.20%, respectively. Devranjan et al. (2017); Gopalan et al. (1989); Kantha et al. (1987) and Girish et al. (2012) were reported similar results for ungerminated buckwheat flour (7.80%), finger millet (3.6%), black gram seeds (4.4%) and black gram flour (5.56%), respectively. The differences in fibre content might be attributed to the removal of seed coat partially during sieving of flour (Wani et al., 2013). In the present study, the were dehulled/ dehusked grains after germination may be the reason for reduced fibre content.

All three grains had significantly different total carbohydrates content. GFMF had highest carbohydrate content of 69.17 % as followed by GBF (68.60%) and GPDF (53.63%).

On comparison between GBF, GFMF, and GPDF; GPDF has good amount of fiber, protein, and ash, but low content in fat. Moreover the chemical composition of finger millet is influenced by cultivar, climatic conditions, stage of maturity and many agro climatic conditions.

1.7. Functional properties of raw materials

Kinsella and Melachouris (1976) defined functional properties as "those physical and chemical properties that influence the behavior of proteins in food systems during processing, storage, cooking and consumption". The functional properties of flours like bulk

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Water absorption capacity

density, dispersibility, WAC, OAC, EA, FC, SC and SI play vital role in food product formulation and product development. Hence those properties were determined for GBWF, GFMF and GPDF, and reported in Table 2.

Bulk Density

Bulk density is a very important parameter in the production of expanded and formed food products. Bulk density of GPDF was 0.83 g/mL, higher than that of GFMF (0.78 g/mL) and GBWF (0.75 g/mL). In addition, bulk density of flours was significantly decreased when the flours were made after germination of grains. Many observations on lowered BD on germination were reported by Akubor and Obiegbuna (1999) for millet flour, Elkhalifa and Bernhardt (2010) for sorghum flour, Bello et al. (2018) for maize flour and Ghavidel and Prakash (2006) for green gram, cowpea, lentil and Bengal gram. Bulk density is a measure of heaviness of flour and is generally affected by the particle size and the density of the flour. The high bulk density of flour suggests their suitability for use in food preparations. On contrast, low bulk density would be an in the formulation advantage of complementary foods (Akpata & Akubor, 1999).

Dispersibility

The dispersibility of a mixture in water indicates its reconstitutability. The higher dispersibility, the better is the reconstitution property (Kulkarni et al., 1991). The percentage dispersibility GBF, GFMF, and GPDF were found to be 76.16, 79.83 and 51.00% (Table 2). GFMF contained highest amount of dispersibility value in all the flours. GPDF had a very poor dispersibility value (only 51%). The major factors controlling dispersibility are temperature, pH, ionic composition and degree of agitation of the solvent. This property is a means of comparing the solubility of a protein in water and hence on germination higher values of dispersibility were observed than the reported values for same grain flours without germination. The value of dispersibility may help to produce fine constituent dough during mixing (Olapade et al., 2014).

The water absorption capacity (WAC) of GPDF (2.4 mL/g) was found to be higher than that of GBF (1.5 mL/g) and GFMF (1.47 mL/g). The lower WAC of GBF could be attributed to the presence of lower amount of hydrophilic constituents in GBF (Akubor & Badifu, 2004). Water absorption capacity represent the ability of a product to associate with water under conditions where water is limited (Singh, 2001). The major chemical compositions that raise the WAC of flours are proteins and carbohydrates, because these constituents contain hydrophilic parts, such as polar or charged side chains (Hodge & Osman, 1976 & Pomeranz, 1985). Germination also increased the water absorption capacity as reported for sorghum flour (Elkhalifa & Bernhardt, 2010), cowpea, green gram, lentil and bengal gram (Ghavidel & Prakash, 2006; Padmashree et al., 1987 & Rosario & Flores, 1981).

Oil absorption capacity

Oil absorption capacity (OAC) is another important functional property of flours, because it plays an important role in enhancing the mouthfeel and retaining the flavour. The oil absorption capacity (OAC) was observed highest in GBF (2 mL/g) followed by GPDF (1.97 mL/g) and GFMF (1.73 mL/g). The water and oil binding capacity of food protein depends upon the intrinsic factors like amino acid composition, protein conformation and surface polarity or hydrophobicity. In addition germination also increased the oil absorption capacities. Many studies showed that the germination increased the oil absorption in flour germinated millet Akubor and Obiegbuna (1999), cowpea, green gram, lentil and bengal gram (Ghavidel & Prakash, 2006) and sorghum flour Elkhalifa and Bernhardt (2010).

Emulsifying activity

The efficiency of emulsification by flour varies with the type, concentration and solubility of the proteins (Achinewhu, 1982). Highest EA (50.3%) was observed in GPDF followed by GBF (49.5%) and GFMF (48.9%). Difference in the EA of protein may

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be related to their solubility exhibited the lowest emulsifying activity and highest emulsion stability. The capacity of protein to enhance the formation and stabilization of emulsions is important for many applications in food products like cake, coffee whiteners and frozen desserts. In these products, varying emulsifying and stabilizing capacity are required because of their various compositions and processes (Adebowale et al., 2005). Germination may cause partial denaturation and may increase the EA due to increased hydrophobicity giving a positive correlation between emulsifying activity and surface hydrophobicity (Wang & Kinsella, 1976).

Foam capacity

Foam capacity refers to the amount of interfacial area that can be created by the

protein (Fennama, 1996). The foam capacities of GBF, GFMF, and GPDF were ranged between 108 to 115%. Paheli dal had contained highest amount of foaming value among all the flours. Several authors have suggested a direct relationship between FC and nitrogen solubility of leguminous flours (Nath & Rao, 1981 & Narayanan & Narasinga Rao, 1982). Chau and Cheung (1998) reported foaming capacity of different legume flours at different pH varied from 64.4 to 140%.

Swelling capacity

The swelling capacity of GPDF had significantly higher than GBF and GFMF. Swelling index describes of stickiness of the resultant product (Oluwalana, 2014). Swelling index of black gram flours ranged from 1.7 to 2.73 g/g (Table 2).

 Table 1: Proximate composition of germinated buckwheat flour (GBF), finger millet flour (GFMF) and naheli dal flour (GPDF)

punch dur hour (Gr Dr)					
Parameters (%)	Buckwheat	Finger millet	Paheli Dal		
Moisture	12.10±0.017 ^a	$11.21 \pm 0.010^{\circ}$	11.50±0.053 ^b		
Protein	$8.20 \pm 0.300^{\circ}$	12.17 ± 0.440^{b}	$23.43{\pm}0.470^{a}$		
Ash	2.11 ± 0.1905^{b}	2 ± 0.040^{b}	$3.83{\pm}0.289^{a}$		
Fat	2.62 ± 0.0763^{a}	$2.5{\pm}0.055^a$	$2.13{\pm}0.082^{b}$		
Crude fiber	$5.80{\pm}0.050^{a}$	$3.5{\pm}0.090^{\circ}$	5.20 ± 0.061^{b}		
Carbohydrates*	69.17 ± 0.200^{a}	68.60 ± 0.580^{a}	$53.63{\pm}0.420^{b}$		

Note: The values are mean \pm S.D of three replications. The values with different superscripts in a row differ significantly (p \leq 0.05) with each other determined by Duncan's tests.

* Calculated by difference method

 Table 2: Functional properties of Buckwheat flour (GBF), Finger millet flour (GFMF) and Paheli dal flour (GPDF)

nour (Grbr)				
Properties	Buckwheat	Finger millet	Paheli dal	
Bulk density (g/mL)	$0.750 \pm 0.016^{\circ}$	0.78 ± 0.018^{b}	0.83±000 ^a	
Dispersibility (%)	76.167 ± 0.764^{b}	79.83 ± 0.764^{a}	51 ± 1.000^{c}	
WAC (mL/g)	$1.500{\pm}0.050^{b}$	$1.47{\pm}0.052^{b}$	$2.4{\pm}0.289^{a}$	
OAC (mL/g)	2.000 ± 0.050	1.73±0.260	1.97 ± 0.052	
Emulsifying activity (%)	0.495 ± 0.010	0.489 ± 0.015	0.503 ± 0.010	
Foam capacity (%)	111.000 ± 2.646^{b}	$108{\pm}1.000^{b}$	$115{\pm}2.000^{a}$	
Swelling capacity (g/g)	$3.200{\pm}0.087^{c}$	$3.94{\pm}0.045^{\text{b}}$	$6.38{\pm}0.104^{a}$	
Swelling Index (g/g)	$1.930{\pm}0.052^{b}$	$1.7 \pm 0.086^{\circ}$	$2.73{\pm}0.075^{a}$	

Note: The values are mean \pm S.D of three replications. The values with different superscripts in a row differ significantly (p \leq 0.05) with each other determined by Duncan's tests.

WAC= Water absorption capacity; OAC= Oil absorption capacity

Buckwheat	Paheli Dal	Finger millet
¥	Ļ	Ļ
Cleaning & washing	Cleaning & washing	Cleaning & washing
¥	Ļ	Ļ
Soaking (36 h)	Soaking (10-12 h)	Soaking (24 h)
	Ļ	Ļ
Germination (48 h) \downarrow	Germination (12 h)	Germination (24 h)
Washing	\checkmark	¥
+	Washing	Washing
Drying $(55 \pm 5^{\circ}C \text{ for})$	¥	¥
8-10 h)	Drying $(55 \pm 5^{\circ}C \text{ for})$	Drying $(55 \pm 5^{\circ}C \text{ for})$
+	8-10 h)	8-10 h)
De-hulling	¥	¥
\downarrow	Milling	Milling
Milling	\downarrow	↓ [_]
	Sieving (300 µm)	Sieving (300 µm)
Sieving (300 µm)	Ţ	Ţ
↓ Packaging	Packaging	Packaging
(a)	(b)	(c)

Fig. 1: Procedure for preparation of flour from germinated seeds

CONCLUSIONS

The finding concluded that significant differences were observed amongst physical, chemical and functional properties of flours obtained from GBF, GFMF, and GPDF. The results of proximate composition, bulk density, dispersibility, WAC, OAC, emulsifying, foaming and swelling properties showed that the germination improved the nutritional properties and functional behaviour of the flours individually. Hence, it seems possible to utilize these flours for further value addition into new food products such as convenience food, weaning food, baked foods and will also suit for allergic persons (gluten tolerant).

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