Effect of Germination And Dehydration on Physical Properties of Horse Gram (Macrotyloma Uniflorum) Flour

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Abstract: Horse gram (Macrotylomauniflorum) seeds were germinated for 24, 48, and 72 hours and further dehydrated using fluidized bed drier for a duration of 3 h at 50°C and force convectional tray drier for 12 h at 50°C to determine the effect of germination and dehydration on the physical properties of horse gram flour. Germination of horse gram flour significantly (p < 0.05) increased the rate of swelling capacity, foam capacity, foam stability, water and oil absorption capacities and decreased the bulk density (4 %), emulsion capacity (9.62 %) and emulsion stability (4.91 %) of the flour. The germination and dehydration of horse gram significantly impacted the peak viscosity, holding viscosity, and set back viscosity of the flour which reflected on the color values as well. The L*, a* and b* values exhibited significant (p < 0.05) difference due to varying germination periods and drying methods. Generally, horse gram flour subjected to germination and dehydration had a beneficial effect on the physical properties measured. The flour germinated for 24, 48 and 72 hours can be used in formulating a range of fabricated and designer food products.

Keywords: Macrotylomauniflorum, germination, dehydration, physical properties, pasting properties

I. Introduction

Inclusion of legumes in daily diet provides numerous beneficial physiological effects in combating various metabolic disorders such as diabetes mellitus, coronary heart disease and colon cancer etc., (Simpson et al., 1981). Horse gram, an underutilized legume, is gaining more attention as legume food due to its high protein content and other nutrients as well (Shasi Jain, 2012). Horse gram, (*Macrotylomauniflorum* (Lam.) Verdcourt (Syn., *Dolichosuniflorus* Lam., *Dolichosbiflorus*auct. non L.)) an underutilized pulse crop native to Southeast Asia has been recognized as potential food source by National Academy of Science (1979). Horse gram is popularly called "Madras Bean" due to the principal cultivation and production of this legume, especially in rural areas, in the erstwhile Madras Presidency state, India, before the States were separated (Chinnasawamy, 2010). It is also known as the "poor man's pulse crop" which is extensively used in traditional and ayurvedic medicines to reduce body weight and to treat diseases like jaundice, urolithiasis, skin disorders etc., (Kumar et al., 2011 & Femina et al., 2012). Due to its dense nutritional profile, new food products, derived from horse gram, are being formulated to serve as supplementary food for the nutritionally vulnerable communities.

Although the use of raw horse gram and its flour as food is well known in recent days, maximum utilization is lacking due to the presence of antinutritional factors like tannin, trypsin inhibitor, phytic acid and bowman-birk inhibitors (Kumar et al., 2002) which interfere with the bioavailability of nutrients present in horse gram. However, notable progression has been achieved through dehulling, germination, fermentation, dehydration, soaking and partial hydrolysis of proteolytic enzyme to reduce the antinutrient factors and to enhance the nutritive value and functional properties of legumes (Deshpande et al., 2002 and Oloyo, 2004). Seed germination process triggers the enzymatic activity of sprouting seeds, which further breaks the carbohydrates, proteins and fats into simpler forms (Nout & Ngoddy, 1997). The process of germination is found to be decreasing the level of polyphenols, oxalic acid and phytic acid present in the horse gram seeds (Sudha, 1995). Further, the use of flour that is produced after germination and dehydration as ingredients for food processing is dependent on its physical and functional properties (Hing, 1990). In order to transform any dry legume into a novel product, analysis of its physical properties is essential to promote the acceptability of the end product. Research, carried out so far, on the effect of germination of horse gram at different time intervals and different dehydration techniques has been sporadic. Hence, the present study was designed to analyze the effect of germinating the seeds at three different germination periods and dehydrating the seeds using two different drying methods on the physical properties of horse gram flours which would further be utilized for production of varied range of novel products.

2.1. Samples

II. Materials And Methods

Horse gram (*Macrotylomauniflorum*) seeds (brown) were procured from a whole sale rice merchant, Puducherry in a bulk quantity to ensure uniformity of the raw material throughout the study and the seeds were cleaned manually to remove the mud particles and dust present along with horse gram.

2.2. Germination

The germination process includes soaking of about six hundred grams of clean seeds in sodium hypochlorite solution (0.07%, w/v) with seed water ratio 1:5 (w/v) in dark condition for 30 min at room temperature in order to remove the surface contaminants present if any (Figure 1). The seeds were further washed with distilled water until neutral pH was achieved and soaked in distilled water (1:5 w/v) for 5 hours at room temperature. The excess water was drained and the seeds were divided into three portions (200 gm each), layered on trays separately and covered using wet cloth to reduce the water evaporation rate. Further, all the three sets were allowed to germinate for 24 h, 48 h and 72 h respectively in incubator at 20 °C. The germination process was evaluated visually with the percentage of germinated seeds.

2.3. Dehydration

Two dehydration techniques namely force convection tray drying (FCTD) ('Nova' digital tray drier-Model: DTD-1203191) and fluidized bed drying (FBD) (5 kg-Model:1141) were used for drying the seeds (Figure 1). The germinated horse gram seeds were divided into two sets and subjected to FCTD and FBD for 12 h at 50 °C and 3 h at 50 °C respectively. Dehydrated horse gram seeds were milled into fine flour with a sieve size 0.25mm and stored in refrigerator at 4°C in separate air-tight containers for further quality analysis.

2.4. Bulk density

Bulk density of the processed horse gram flour was determined as per the method described by Okaka and Potter (1977). About 50 gm of processed horse gram flour was filled into a 100 ml graduated measuring cylinder and tapped gently for several times on the flat surface of a wooden table until it reaches a constant volume. The reading was taken and the bulk densities of triplicate values were read as g/ml.

2.5. Swelling capacity

Swelling capacity was determined by the method described by Leach et al., (1959) with modification for small samples. In a graduated centrifuge tube, one gram of sample was mixed with 10 ml of distilled water and heated at 80°C for 30 min in a shaking water bath. The suspension was centrifuged as 1000 x g for 15 min after heating. The weight of the paste was taken after decanting the water. The swelling capacity was calculated using the following formula.

Swelling capacity = Wt. of the paste Wt. of the dry flour

2.6. Water and oil absorption capacities

Water/oil absorption capacity was determined following the method of Beuchat (1977). About a gram of processed horse gram flour was mixed well with 10 ml of distilled water/ oil in a centrifuge tube with the help of vortex mixer and it was allowed to remain for 1 hour in room temperature. Later, the mixture was centrifuged at 3000 rpm for 30 minutes and the water absorption capacity was calculated as ml/gm.

2.7. Emulsifying activity and stability

x 100 -

Emulsifying properties of processed horse gram flour were determined using the method outlined by Yasumatsu et al., (1972). About 0.5 gm of processed horse gram flour was mixed well with 3 ml of distilled water followed by 3 ml of oil in a graduated centrifuge tube and shaken vigorously by hand for 5 min. The mixture was centrifuged at 2000 x g for 30 min. The volume of emulsified layer divided by that of whole slurry after centrifugation and multiplied by 100 was recorded as the emulsifying activity of the flour.

Emulsifying activity =

Height of emulsion layer

Height of whole slurry

The same slurry was heated at 80 °C for 30 min and cooled to room temperature to determine the emulsion stability which was calculated using the following formula.

Height of emulsion layer after heating

Emulsifying activity =

Height of whole slurry

x 100

2.8. Foam capacity and foam stability

Foaming property was determined according to the method of Okaka and Potter (1977). One gm of processed horse gram flour was dispersed in 50 ml of distilled water in the screw capped test tube and it was shaken vigorously for 5 min. The mixture was immediately transferred to a 250 ml graduated cylinder. The volume of foam formed was noted as the foam capacity (ml/100ml). Foam stability was recorded after one hour by observing a fall in the formed foam of the sample (ml/100ml).

2.9. Color

Color of processed horse gram flour was analysed using a Hunter color Lab colorimeter (Model:CX2748, East Match QC, V 4.0, Hunter lab, USA). In the colorimeter, the sample color is denoted by three dimensions (L*, a* and b*) where L* indicates the measure of lightness of the flour from 100 for perfect white to zero for black. The dimensions a* and b* denote the redness/greenness and yellowness/blueness of the flour respectively.

2.10. Pasting properties

Pasting properties of processed horse gram flour were determined using a Rapid ViscoAnalyser (Starchmaster 2, Newport Scientific Pvt. Ltd, Australia, N13713, V3.2). A 3 gm flour sample was mixed thoroughly with 25 ml of water in canisters and placed in the Rapid Visco Analyser. Readings were recorded for pasting temperature, peak viscosity, holding viscosity, final viscosity, breakdown viscosity and set back viscosity.

2.11. Data analysis

Analysis of Variance (ANOVA) and Duncan's multiple range test (p<0.05) of the data obtained were performed using statistical software SPSS 18.0 (SPSS Inc, Chicago, USA). The data presents the mean with standard deviation of triplicate values.

III. Results

3.1. Bulk density

The bulking property of a powder alters according to the preparation methods, different treatments administered and storage (WHO, 2012). The density of the processed products or the uniqueness of its container determines the amount and strength of packaging material (Wilhelm *et al.*, 2004). In this study, gradual increase in germination periods showed decrease (p < 0.05) in the bulk density of horse gram flour (Table 1). Germination of horse gram flour at 24 h, 48 h and 72 h significantly reduced the bulk density of horse gram flour by 4 % (0.69 – 0.74gm/ml) when compared to the control sample (0.75 g/ml). The effect of drying techniques on horse gram flour after 24 h and 72 h germination was less significant than on the sample obtained after 48 h germination. This could be due to decrease in heaviness and dispersability of the processed flour samples as observed by Chinma et al., (2009) for yellow tiger nut flour. This result is in agreement with Akubor and Obiegbuna (1999) who observed lesser bulk density value for germinated millet flour. Similarly, Ghavidel and Prakash (2006) reported reduced bulk density for germinated green gram, cowpea, lentil and bengal gram. As an effective traditional technology, germination enhances the feasibility of incorporation of horse gram flour in weaning foods (Malleshi, Daodu and Chandrashekar, 1989).

3.2. Swelling capacity

Swelling capacity is the volume of expansion of molecule due to the consumption of water up to a level where the colloidal suspension is complete (Ayernor, 2002). In addition, bonding forces and starch species present in the flour determine the degree of swelling and solubility of the flour. Table 1 depicts the effect of germination and dehydration on swelling power. Germination of horse gram at 24 h, 48 h and 72 h significantly (p < 0.05) increased the swelling capacity of horse gram flour when compared to the raw horse gram flour (1.62 ml). , Horse gram flour germinated for 24 h and 48 h exhibits significant difference in the drying methods (1.50 ml & 1.56 ml and 1.61 ml & 1.68 ml) than the flour obtained after 72 h germination (Table 1). The presence of carbohydrates in horse gram and weakening of the intra-granular binding forces of the starch granule results in minimum restriction to swelling thereby increasing the swelling power of the flour (Wang et al., 2011). The result is in acceptance with that of Ocheme and Chinma (2008) for increased swelling capacity of germinated millet flour. The processed horse gram flour has higher swelling capacity due to starch gelatinization and hence it is more appropriate for the preparation of extruded snack foods.

3.3. Water Absorption Capacity

Water absorption characteristics symbolize the ability of a product to associate with water under conditions where water is restrictive and considered to be an index of the capability of protein to absorb and

retain water. Water absorption capacity of germinated horse gram flour shows an increasing trend as given in Table 1. Germination process for 24 h and 48 h with force convection tray drying and fluidized bed drying (1.50 ml and, 1.56 ml, 1.61 ml & 1. 68 ml) significantly increased (p < 0.05) the water absorption capacity of horse gram flour. However, horse gram flour germinated for 72 h and subjected to two different drying techniques exhibited similar values. It can be assumed that the polar amino acid residues of proteins with substantial attraction for water molecules could have increased the water absorption capacity of germinated horse gram flour (Sreerama *et al*, 2012). The WAC of germinated horse gram flour is comparatively higher than germinated sorgum flour (131.34 %) as reported by Elkhalifa et al., (2010). Similar observations were already reported by Okezie and Bello (1988) and Obalolu and Cole (2000). Since flour with high WAC possibly becomes a noble ingredient in bakery usage by promoting freshness and handling features, germinated horse gram flour is useful in bread formulations.

3.4. Oil Absorption Capacity

Oil absorption capacity (OAC) of legume flours is an essential property to develop novel food products and store them for a long period. Flavor and mouth feel of food depends on the fat molecules present in the flour to some extent (Kinsella, 1976). The oil absorption capacity of germinated and dehydrated horse gram flour exhibited increasing trend from 0.793 to 1.836 ml/gm (Table 1). Irrespective of the drying techniques adopted, oil absorption capacity of the horse gram germinated for 24 h showed slight decrease than that of the raw flour. On the other hand, OAC of germinated horse gram flour for 48h and 72h exhibited significant increase (p<0.05) from 1.15ml/gm to 1.83 ml/gm than the control sample and the sample obtained after 24 h germination. Surface availability of hydrophilic amino acids and other non-polar amino acid chains as dietary components in the processed horse gram are the major causes for the oil absorption property of flour. Decreased fat content of horse gram upon germination could also be attributed to the increase in consumption of oil in its structure. The result is in excellent agreement with the reports outlined by Elkhalifa et al., (2010) for germinated sorghum flour and Chinma et al., (2009) for germinated tiger nuts. Therefore, germinated horse gram flour finds applications in the preparation of emulsion type food products with enhanced mouth feel (Kinsella, 1976).

3.5. Emulsion Capacity & Stability

The emulsifying property is an important parameter of legume flours which varies with the type, protein solubility and concentration of proteins present in that legume (Achinewhu, 1986). The study reveals that emulsion capacity and stability of raw and germinated horse gram flour (Figure 2) increased with germination time. Emulsion capacity and stability of horse gram flour after 24 h, 48 h and 72 h germination was significantly less (p < 0.05) than those of raw horse gram flour (40.56%). However, dehydration techniques did not show any significant difference in emulsion capacity and stability of the flour obtained after 24 h, 48 h and 72 h germination. Decrease in emulsion property observed might be due to the hydrophobicity or hydrophilicity ratio of protein, protein contents and its structural constraints forming a film around the dispersed oil droplets through unfolding by itself (Boye et al., 2010). Similar reduction in emulsion activity after germination was reported by Gamel et al., (2006) for germinated amaranth seeds flour by 20-21% and Cabrejas et al., (2008) for cowpea (9%), jack bean (11%), dolichos (5%) and mucuna (9%) seeds. The emulsion formation and stabilization capacity of horse gram flour may be useful in the formulation of bakery products, coffee whiteners and frozen desserts that require these particular capacities in different compositions.

3.6. Foam Capacity and Stability

The foam capacity (FC) and foam stability (FS) are determined by the liquid loss resulting from destabilization 'leakage' that is measured as a volume decrease. The foams of legume flours are reported to be dense with low foam volume but with relatively high foam stability. Foam capacity and foam stability values recorded for germinated and dehydrated horse gram flour differ significantly (p<0.05) (Figure 3). Irrespective of the drying techniques adopted, the foaming properties of horse gram flour germinated at 24 h, 48 h and 72 h exhibited increased values when compared to the non-germinated horse gram flour (FC = 52.66 ml & FS = 46.50 ml). Significant increase in FC and FS of processed horse gram flour might be due to the dissimilarity in protein and its concentration too. The nature and quantity of horse gram protein also influenced the foaming stability of the flour. The values are in accordance with the reports described by Ghavidel and Prakash (2006) for germinated cow pea, lentil, green gram and bengal gram. Greater increase in the surface area of the liquid or air interphase lead to the effective denaturation of proteins and their aggregation during whipping of germinated horse gram flour. Owing to this significant property, germinated horse gram flour could be useful in the formulation of cakes and biscuits with enhanced textural quality (Blitz and Grosch, 1999).

3.7. Color analysis

Color is essential for the acceptability and final quality of a food product. The color values of germinated and dehydrated horse gram flour are presented in Table 2. Horse gram flour germinated at 24 h, 48 h and 72 h subjected to two different drying techniques showed significant difference in L* values (82.91, 82.13, 81.32, 83.63, 82.39 & 81.32) than the control sample which was little darker due to the dark brown seed. Regarding a* values, irrespective of the drying methods adopted, germinated horse gram flour exhibited positive values. This indicates that the horse gram is towards the milder side of red color which is contributed by the brown husk color after processing. However, a* values of convectional tray dried flour (2.24) and fluidized bed dried flour (2.29) germinated for 72 h recorded similar values close to control flour (2.29). Germination and exposure of the inner starchy material enhanced the lightness of the flour whereas pretreatments and drying techniques decreased it. On the other hand, germination reduced the redness while drying treatment increased it. (Shingare &Thorat (2013). Significant difference in b* values between the samples might be due to the amount of carbohydrate and protein of horse gram (Jamin & Flores 1998). Factors like difference in the colored pigments of the flours, composition of the flours and botanical origin of the plant might contribute to different color characteristics of different flours (Aboubakar et al., 2008). Germination significantly increased the total color difference (dE) of horse gram flour subjected to both drying methods adopted in the study. As the germination degree deepens, formation of more starch and protein hydrolysis results in maillard reaction during drying showing lower L*, higher a*, b* and dE values than that of raw flour. The values had good acceptance with respect to the color values as reported by Tian et al., (2010) for germinated oat flour. Similarly, Hunter color L*, a* b* and dE values of 81.64 to 86.41, -0.72 to -1.10, 14.1 to 20.7 and 64.18 to 66.96 respectively were reported by Kaur and Singh (2005) for chickpea flours.

3.8. Pasting properties

The results of Rapid Visco Analyser (RVA) of raw and germinated horse gram flour are presented in Table 3. Both germination and dehydration significantly influenced the pasting properties of horse gram flour. Germination of horse gram for 24 h, 48 h and 72 h showed significantly (p < 0.05) less pasting temperature values than dehydration techniques. Peak viscosity of the flour with any given concentration is an important characteristic of a starch. Peak viscosity of horse gram flour germinated for 48 h subjected to force convection tray drying (753.00cP) reflects better pasting property. Final viscosity of unprocessed horse gram flour (1150.33 cP) was higher than processed horse gram flour (336.33 cP - 520.66 cP). Similarly, holding viscosity and set back viscosity of the germinated horse gram flour recorded significantly lower values than the raw flour with slight difference between the force convection tray drying and fluidized bed drying. Syneresis of flour starch upon cooling of cooked starch pastes gives the set back viscosity (Singh et al., 2004). Significant difference in viscosity values could be attributed to the aggregation of the amylase molecules of raw horse gram flour which is absent in processed horse gram flour (Botham et al., 1995). In addition, solubility of the flour and presence of broken granules which absorb more water also influence the viscosity of the dispersion. Therefore, germinated horse gram flour could be extensively used in the development of extruded food products. This result is in good agreement with the report given by Kaur (2007) who observed a rapid increase in viscosity when an adequate number of granules become swollen in the flour sample.

IV. Conclusion

Germination and dehydration of horse gram seeds influenced the physical properties of the flour. Acceptable pasting properties of germinated and dehydrated horse gram flour than the raw flour may find better application in the novel food formulations like convenience foods including ready to eat snacks and ready to cook products. Significant changes induced by germination would make the horse gram as one of the suitable legume food to be incorporated in the daily diet of all age group people. It is highly essential to carry out innovative marketing strategies among consumers to utilize the food products and educate the public with regard to their beneficial aspects in addition to the development of technologies for the manufacture of germinated horse gram based food products.

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Horse gram flour		Bulk density (gm/ml)	Swelling capacity (%)	Water absorption capacity (ml)	Oil absorption capacity (ml)	
Raw		0.75±0.01 ^a	$2.29{\pm}0.10^{d}$	1.62±0.05 ^{bc}	1.13±0.09 ^{bc}	
24 hrs	TD	0.73±0.03 ^{ab}	2.08 ± 0.06^{b}	1.50 ± 0.05^{d}	0.79±0.03 ^c	
germinated	FBD	$0.74{\pm}0.02^{a}$	1.96±0.15 ^a	1.56±0.02 ^{cd}	$0.83 \pm 0.70^{\circ}$	
48 hrs	TD	0.72±0.01 ^{abc}	2.16±0.30 ^{bc}	1.61±0.05 ^{bc}	1.15±0.07 ^{bc}	
germinated	FBD	0.72±0.01 ^{abc}	2.06±0.36 ^{ab}	1.68 ± 0.06^{ab}	1.55±0.40 ^{ab}	
72 hrs	TD	0.69±0.00 ^c	2.24±0.65 ^{cd}	1.77±0.05 ^a	1.83±0.13 ^a	
germinated	FBD	0.70±0.01 ^{bc}	2.29±0.73 ^d	1.77±.007 ^a	1.60 ± 0.48^{ab}	

 Table 1. Physical properties of germinated and dehydrated horse gram flour

Table 2. Color values of germinated and dehydrated horse gram flour

Horse gran	n flour	L*	a*	b*	dE
Raw	7	82.34±0.64bc	2.29 ± 0.10^{d}	11.69±0.26 ^b	-
24 hrs	TD	82.91±0.20 ^{ab}	2.08±0.06 ^b	11.22±0.07°	0.31±0.09°
germinated	FBD	83.63±1.01 ^a	1.96±0.15 ^a	10.63±0.16 ^a	1.19±0.20 ^b
48 hrs	TD	82.13±0.46b ^{cd}	2.16±0.30 ^{bc}	11.58±0.70 ^b	0.95±0.03 ^b
germinated	FBD	82.39±0.45 ^b	2.06±0.36 ^{ab}	10.87 ± 0.14^{a}	0.88 ± 0.06^{b}
72 hrs	TD	81.32±0.38 ^{cd}	2.24±0.65 ^{cd}	12.63±0.03 ^a	$2.14{\pm}0.32^{a}$
germinated	FBD	81.16±0.75 ^d	2.29 ± 0.73^{d}	11.57±0.71 ^a	2.18 ± 0.67^{a}

TD – Force convection tray drying, FBD – Fluidized bed drying. Values are means of three replicates \pm standard deviation. Values in the same column with different alphabets are significantly different at p<0.05

Table 3. Pasting properties of raw, germinated and dehydrated horse gram flour

Horse gram flour		Pasting temperature	Peak viscosity (cP)	Holding	Final viscosity	Break	Setback
		(min)	viscosity (cr)	viscosity (cr)	(er)	viscosity (cP)	viscosity (ci)
Raw		82.60 ± 0.26^{a}	1015 ± 24.00^{a}	865.00±28.16 ^a	1150.33±58.02 ^a	147.66±3.78 ^a	285.33±29.8ª
24hrs	TD	80.98±1.25 ^b	626.66±34.15°	376.33±63.82 ^b	520.66±65.20 ^b	191.00±65.64 ^a	144.33±0.57 ^b
germinated	FBD	81.30±0.40 ^{ab}	579.66±11.59 ^d	372.33±22.94 ^b	498.66±21.36 ^b	181.33±32.86 ^a	141.00±22.51 ^b
48hrs	TD	81.36±0.56 ^{ab}	753.00±26.90 ^b	340.00±51.73 ^{bc}	432.66±60.92 ^b	192.33±16.01 ^a	90.66±10.06 ^c
germinated	FBD	81.96±0.61 ^{ab}	360.66 ± 24.82^{f}	250.33±45.21 ^d	336.33±45.08°	170.33±77.57 ^a	86.00±1.00 ^c
72hrs	TD	81.86 ± 0.98^{ab}	538.66±38.52 ^d	256.00±50.26 ^d	346.00±47.03°	191.00±77.11 ^a	89.66±4.16 ^c
germinated	FBD	82.26±0.32 ^{ab}	411.66±4.93 ^e	265.66±27.30 ^{cd}	518.09±27.70°	179.33±25.81ª	76.33±2.08°

TD – Force convection tray drying, FBD – Fluidized bed drying. Values are means of three replicates \pm standard deviation. Values in the same column with different alphabets are significantly different at p<0.05



Figure 1. Flowchart showing the process for germination and dehydration of horse gram seeds

TD – Force convection tray drying, FBD – Fluidized bed drying. Values are means of three replicates \pm standard deviation. Values in the same column with different alphabets are significantly different at p<0.05



Figure 2. Emulsion capacity and stability (ml) of raw, germinated and dehydrated horse gram flour



Figure 3. Foam capacity and stability (ml) of raw, germinated and dehydrated horse gram flour