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RESEARCH PAPER

Effect of Fermentation and Dehydration on the Nutritional and Functional Properties of Horse Gram (*Macrotyloma uniflorum*) Flour

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Abstract

A study was conducted in order to investigate the effect of fermentation and dehydration on the nutritional and functional properties of horse gram flour. Fermentation was carried out for 24 hrs, 48 hrs and 72 hrs and standard methods were adopted to analyze the nutritional and functional properties of the processed flour. Fermentation period had significantly reduced the carbohydrate, fat, ash but increased the protein, moisture and fiber of horse gram flour. Dehydration techniques did not affect the nutrients present in the processed flour appreciably. But, Fluidized bed drying effectively increased the foam capacity and water absorption capacity of fermented horse gram while fermentation period had decreased the swelling capacity and oil absorption capacity of processed flour, irrespective of the drying methods. Hence, the utilization of fermented and dehydrated horse gram and its flour not only improve the nutrient utilization and bioavailability of nutrients but also upsurge its application in therapeutic food formulations for specific health conditions.

Keywords: Horse gram, germination, fermentation, nutritional analysis, functional properties, dehydration

Legumes and pulses are good sources of dietary fibers especially in their husk fractions, which promote their tremendous therapeutic health effects. In addition to a protein rich source, legume consumption is considered to be useful to reduce a number of diseases like coronary heart disease (Anderson *et al.*, 1984), colon cancer (Hangen, 2002 and Hughes, 1997), diabetes and obesity (Geil, 1994 and Venkateswaran, 2002), prostate cancer (Kolonel, 2000) and breast cancer (Adebamowo, 2005). Horse gram (*Macrotyloma uniflorum* (Lam.) Verdcourt (Syn., *Dolichos uniflorus* Lam., *Dolichos biflorus* auct. non L.)) is an underutilized pulse crop which is native to Southeast Asia and Tropical Africa (Purseglove, 1974 and Smart , 1985). Among underutilized legumes, horse gram has been recognized as potential source of protein and other nutrients (Priyawiwatkul, 1996). Due to the emergence of new food processing technologies, this legume can be consumed as whole, dehulled, splits, canned, boiled, roasted or ground into flour, which occupied a very important place in human diet in many developing countries (Kadam, 1985). The vegetative parts of horse gram plant are good source of vitamins like carotene, thiamin, riboflavin, niacin and vitamin C (Sodani *et al.*, 2004).

Though the legume seeds are good source of nutrients, their use in food and feed is still limited since they contain sulfur containing aminoacids, presence of antinutritional components and low protein digestibility (Mubarak, 2005). The antinutritional factors in legumes include phytic acid, trypsin inhibitor, chymotrypsin, lectins, polyphenols, tannins and flatulence causing oligosaccharides (Reddy 1985; Urbano, 2000; Gupta, 1987; Singh, 1998 and Udensi, 2007). Various processing methods and traditional treatments like soaking, germinating, drying, fermentation and cooking along with other cereals and pulses either completely or partially reduces the toxicity and improves the nutritional quality as well as the bioavailability of minerals and other nutrients (Harmuth-Hoene *et al.* 1987; Reddy, 1985 and Neilson 1991). Fermentation with *Rhizopus oligosporus* is known to eliminate almost completely stachyose, the most flatulent oligosaccharide in soya bean, cow pea and ground bean. Product development based on fermented legumes have increased the functional components like isoflavone aglycones and active peptides having more health benefits (Hong *et al.* 2004). Fermented legume flour could be a good source of protein fortification for a variety of foods products for protein deficient consumers in developing countries as well as functional ingredient for the food industry (Jianmei Yu *et al.* 2007). Hence, the present study was conducted to investigate the effect of fermentation and dehydration on the nutritional and functional properties of horse gram and to enhance its utilization and application in therapeutic food preparations and food industries.

Materials and Methods

Preparation of raw material

Horse gram (*Macrotyloma uniflorum*) was procured from a supermarket, Puducherry in a bulk quantity, cleaned thoroughly to remove the mud particles and other unwanted materials, and stored in plastic containers as it ensures the uniformity of the raw material throughout the study.

Fermentation Process

Horse gram seeds were treated with sodium hypochlorite (0.07% v/v) solution for 30 min to remove the surface contaminants. Then, the seeds were washed with distilled water and drained well. The washed seeds were divided into three equal portions and mixed well with demineralized water in a ratio of 1:5 (w/v), separately. All the three portions were allowed to ferment naturally in separate containers at 30ºC (Nche, *et al.,* 1994). Af er 24^{th} hr, a small amount of fermented water was extracted from the first portion and added to the second and third portions and allowed to ferment for 48 hrs and 72 hrs respectively, in order to obtain an accelerated fermentation by enrichment of acidifying microbiota (Jeroen *et al,* 2000). Af er the fermentation process, the seeds were drained and subjected to dehydration process. Complete processing of horse gram seeds is given in figure 1.

Dehydration Methods

Two different types of dehydration techniques viz. forced convection tray drying and fluidized bed drying were adopted to dry the horse gram seeds. Each portion of the fermented horse gram was again divided into two parts and subjected to dehydration, separately. The time and temperature maintained for forced convection tray drying ('Nova' digital tray drier-Model: DTD-1203191) and fluidized bed drying (FBD 5 kg-Model:1141) were 15 hrs and 50ºC and 2 hrs and 50ºC, respectively (Fig. 1). The fermented and dehydrated horse gram seeds were milled into fine powder with a sieve size 0.25 mm and stored in refrigerator at 4ºC, in separate air-tight containers for further quality analysis.

Analysis of the Flour

The proximate composition of fermented and dehydrated horse gram flour was analyzed by the standard methods of the Association of Official Analytical Chemists (2003). Functional properties like bulk density (Okaka and Pot er, 1979), swelling capacity (Leach *et al.* 1959), water and oil absorption capacities (Buchet, 1997), foaming capacity and stability (Narayana and Narasinga Rao, 1982) were also determined for the processed horse gram flour.

Statistical analysis

The triplicate values of all the experiments were recorded and analysed statistically using statistical tool SPSS. The data were subjected to ANOVA and independent sample t-test. The significance of mean differences was determined by least significant difference (LSD).

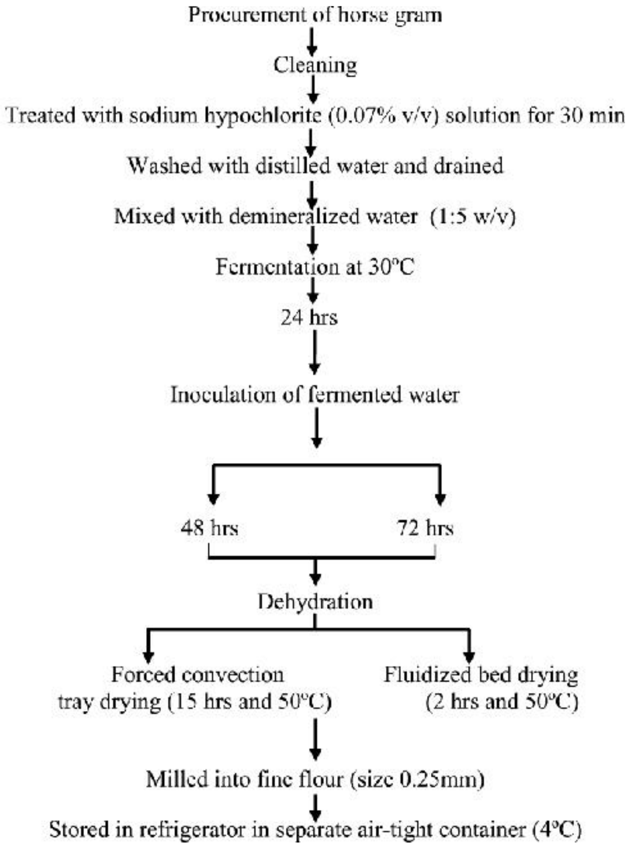

Fig. 1: Flow diagram of process for fermentation and dehydration of horse gram

Results and Discussion

Proximate analysis

Ash

The results of the study showed that the ash content of fermented horse gram flour gradually decreased (Table 1) as the fermentation period increases in the case of forced conventional tray dried flour and the value is more or less similar (1.567% – 1.500%) in fluidized bed dried horse gram flour. Such a significant difference in the values could be at ributed to the leaching of the soluble inorganic salt during fermentation process representing a rough estimation of the minerals present in the product (Fasasi, 2009). This concept is in agreement with the report of Marimuthu *et al.* (2013), for the ash content of raw horse gram flour to be 2.24%.

Moisture

The moisture content (Table 1) of the processed horse gram flour showed a relatively lower value (4.33 -5.33%) on comparing with the moisture content of raw horse gram flour (8.83%) reported by Sreerama *et al.* (2008) and also other common leguminous seeds such as cow pea and kidney bean (10 - 11%) and green pea (7 to 8%) (Khat ab *et al.,* 2009). In addition to that, the moisture content of force convention tray dried horse gram flour (4.33-5.4 %) was lesser than the fluidized bed dried flour (5.3-5.5%). The moisture content of fermented horse gram flour increased with increase in fermentation time in both the drying methods. This might be due to the increased dry mat er content in low fermented flour. The moisture content is described to have a significant influence on the overall quality and shelf-life of legume seeds (Bhat *et al.,* 2008). Food that contain low moisture usually slow down the growth of microorganisms and promotes the shelf-life of the food material.

Carbohydrate

The carbohydrate content (Table 1) of fermented and dehydrated horse gram flour was in the range of 60.67g to 68.4 g/100g which is similar to the carbohydrate content of raw horse gram (66.6 g/100g) as reported by Sreerama *et al.* (2012). As the fermentation period increases, the carbohydrate content of the flour decreases in both drying methods, but the reduction was comparatively greater in forced conventional tray dried horse gram flour than other dried flour. This significant difference in values could be at ributed to the utilization of major portion of soluble sugars in the dry seeds for the respiratory activity during fermentation process. High carbohydrate foods from plants that are highly processed or refined could be used as a food sources and also utilized well in the preparation of sweets, cookies, candies etc.

Table 1: Proximate analysis of fermented and dehydrated horse gram flour

Nutrients	HGF1FC (24 hrs)	HGF2FC (48 hrs)	HGF3FC (72 hrs)	HGF1FB (24 hrs)	HGF2FB (48 hrs)	HGF3FB (72 hrs)
Ash	2.133±0.306[a]	1.800±0.211[a]	1.66±0.231[b]	1.600±0.173[b]	1.567±0.115[a]	1.500±0.346[b]
Moisture	4.33±0.29[a]	5.40±0.17[b]	4.57±0.12[a]	5.40±0.17[a]	5.50±0.00[a]	5.33±0.29[a]
Carbohydrate	66.23±1.27[a]	64.23±0.86[b]	60.93±1.10[b]	68.40±2.26[a]	66.13±0.91[b]	60.67±0.66[b]
Protein	14.26±0.91[a]	16.03±0.14[b]	17.91±0.06[c]	15.50±0.33[a]	17.18±0.34[b]	18.41±0.10[c]
Fat	1.13±0.12[a]	1.07±0.12[a]	1.13±0.12[a]	1.20±0.00[a]	1.07±0.12[a]	1.13±0.12[a]
Fiber	2.47±0.06[a]	3.33±0.12[b]	3.53±0.12[c]	2.87±0.12[a]	3.70±0.00[b]	3.77±0.42[b]

Means with the same superscript (a, b, c) within the same row do not differ significantly ($P > 0.05$)

HGF1FC-24hrs fermented force convection tray dried horsegram flour, HGF2FC-48hrs fermented force convection tray dried horsegram flour, HGF3FC-72hrs fermented force convection tray dried horsegram flour, HGF1FB-24hrs fermented fluidized bed dried horsegram flour, HGF2FB-48hrs fermented fluidized bed dried horsegram flour, HGF3FB-72hrs fermented fluidized bed dried horsegram flour

Protein

It is evident from the study that the protein content of fermented and dehydrated horse gram increases as the fermentation period increases and the values are within the range of 14.26 to 18.41 % where the drying methods does not affect the protein values much (Table 1). It is a well known fact, that the food legumes are naturally high source of proteins (Deshpande, 1992) and in addition to that, fermentation increases the protein content due to the removal of husk from the endosperm of the seeds. This concept is in agreement with the report given by Khadam *et al.* (1989) that dehulling of legume seeds significantly increases the level and availability of protein since greater weight of cotyledons retains the protein of the whole seed. Furthermore, there is an apparent increase in protein through respiration of carbohydrates. The high protein content of processed horse gram highlights its usage in diet for malnutrition and in the formulation of protein rich ready to cook and ready to eat snack foods.

Fat

The fat content of fermented horse gram flour is represented in the table 2. The fat values of fermented and dehydrated horse gram flour increased (1.07 to 1.2%) with increase in the fermentation time and the dehydration techniques had no influence on the fat content much. However, the fat content of raw horse gram seed as observed by Sreerama *et al.* (2012) and Marimuthu *et al.* (2013) were 1.4% and 1.25%, respectively, which is higher than those obtained in the present study result indicating the concept that fermentation decreases the legume fat. The decrease in fat content might be due to the enhanced lipolytic enzyme activity during fermentation promoting the hydrolyses of fat and converting it into fat y acid and glycerol (Chinma *et al.*, 2009). Lower fat content of fermented flour will promote the shelf-life of the flour by quashing the rancidity and contributes to the low energy value of the sample, which is also evidenced by the lower carbohydrate value of the processed horse gram flour. Patients seeking low fat and weight reduction diets can utilize the fermented and dehydrated horse gram flour in food preparations.

Crude Fiber

The results showed that the crude fiber content of fermented and dehydrated horse gram flour gradually increased (2.47 to 3.53 and 2.87 to 3.77) with increase in fermentation period in both the drying methods. According to the report of Sreerama *et al.* (2008) the total dietary fiber of raw horse gram was 16.3g which is higher than the fermented horse gram flour and this could be at ributed to the sugar

Table 2: Functional properties of fermented and dehydrated horse gram flour

Functional Properties	HGF1FC (24 hrs)	HGF2FC (48 hrs)	HGF3FC (72 hrs)	HGF1FB (24 hrs)	HGF2FC (48 hrs)	HGF3FB (72 hrs)
pH	6.89±0.03[a]	6.53±0.03[a]	6.45±0.21[b]	6.96±0.05[a]	6.68±0.06[b]	6.37±0.19[c]
Bulk density	0.98±0.00[b]	0.506±0.05[a]	0.53±0.22[a]	0.88±0.00[a]	0.58±0.87[a]	0.661±0.22[a]
Swelling Index	4.63±0.02[a]	4.32±0.02[a]	4.27±0.03[b]	4.51±0.05[a]	4.64±0.02[b]	4.27±0.02[c]
Foaming Capacity	7.667±0.577[a]	3.667±0.577[b]	6.333±1.528[a]	11.00±0.000[b]	8.333±1.155[a]	8.667 ± 0.577[a]
Water Absorption Capacity	8.27±0.12[a]	8.40±0.20[a]	7.93±0.12[b]	8.00±0.00[a]	8.33±0.12[b]	8.53±0.12[c]
Oil Absorption Capacity	8.47±0.12[b]	8.53±0.12[b]	8.07±0.12[a]	8.07±0.12[a]	8.33±0.12[b]	8.33±0.12[b]

Means with the same superscript (a, b, c) within the same row do not differ significantly ($P > 0.05$)

HGF1FC-24hrs fermented force convection tray dried horsegram flour, HGF2FC-48hrs fermented force convection tray dried horsegram flour, HGF3FC-72hrs fermented force convection tray dried horsegram flour, HGF1FB-24hrs fermented fluidized bed dried horsegram flour, HGF2FB-48hrs fermented fluidized bed dried horsegram flour, HGF3FB-72hrs fermented fluidized bed dried horsegram flour

utilization for the metabolic activity and degradation of the fiber by the enzymes during the fermentation process (Ikenenbomach *et al.* 1986). However, gradual increase in the fiber content of fermented horse gram along with fermentation time for both the drying methods might be due to the retention of fibrous seed coat, followed by the metabolic activity during fermentation (Oke *et al.* 1985). Presence of more dietary fiber promotes the utilization of this food legume in the supplementation of high residue diets.

Functional Properties

pH

The pH had a significant effect on the fermentation of horse gram flour. The pH of the fermented horse gram flour increased with increase in fermentation period in both the drying methods which is indicated by the decrease in the values (Table 2). pH of fermented flour increases sharply with concomitant increase in the fermentation time, due to the increase in the acidity during fermentation.

Bulk density

The bulk density of fermented and dehydrated horse gram flour (Table 2) ranged from 0.50 g/cm^3 to 0.98g/cm^3, although, there were significant difference *(P>0.05)* among the flour samples with increase in fermentation period irrespective of the drying techniques which does not affect the values much. The values obtained from this study were comparable with the values reported by Adebowale, O.J. *et al.* (2011) for pigeon pea (0.63 to 0.80 g/cm^3) and by Okaka and Pot er (1979) for cowpea (0.60 g/cm^3) which are lower than the fermented horse gram flour. The effect of fermentation on the bulk density of horse gram flour is presented in table 2. According to WHO (2012), the bulking properties of a powder depends on the preparation, treatment and storage. As per Wilhelm *et al.* (2004) the amount and strength of packaging material is determined by the density of the processed products or the characteristics of its container.

Swelling capacity

Swelling capacity (Table 2) has been directly allied with the presence of starch, which indicate the capability to absorb water and increase in size. Starch granules create some differences in their bonding forces indicating further changes in the swelling and solubility of the flour. The starch granules start imbibing water when the bond relaxes during increased thermal agitation causing leaching out of amylase into the aqueous medium (Balagopalan, 1988). The swelling capacity of fermented horse gram flour decreases with increase the fermentation period in both the drying methods. The water holding capacity of the starch molecules by hydrogen bonding is responsible for the swelling power of the flour. During gelatinization, there is a breakage in the hydrogen bonds stabilizing the structure of the double helices in crystallites and the crystalline of starch regulates swelling. Though the processed horse gram flour has higher swelling capacity due to starch geletinization, it is more appropriate for the preparation of extruded snack foods (Wang *et al.* 2011).

Water Absorption Capacity

Water absorption characteristics (WAC) symbolize the ability of a product to associate with water under conditions where water is restrictive. The water absorption capacities of fermented horse gram flours, under ambient conditions, were in the range of 7.93-8.53 ml (Table 2). The result also showed that the WAC of the fluidized bed dried (FBD) fermented horse gram flour was higher than that of forced convention tray dried (FCTD) fermented horse gram flour. These values are comparatively higher than the reported values for different chickpea flours which range from 1.33g to 1.47g (Maninder Kaur *et al.* 2004). Polar amino acid residues of proteins have an attraction for water molecules and alterations in WAC of different legumes could be due to the presence of the amino acids in legumes (Sreerama *et al.* 2011). Flour with high WAC could be a noble ingredient in bakery usages, such as bread formulations, since a higher WAC facilitates bakers to add more water to the dough, thus enhancing the handling features and freshness in bakery products.

Oil Absorption Capacity

The oil absorption capacity of fermented horse gram flour was found to be within the range of 8.07ml to 8.53ml and showed significant differences with the fermentation period irrespective of the drying techniques (Table 2). More hydrophobic proteins exhibit superior binding of lipids, surmising that non-polar amino acid side chains bind the paraffin chains of fats. It could be thus, inferred that fermented horse gram flour, which showed higher OAC, had more accessible non-polar side chains in its protein molecules than chickpea flours. Since food fat act as a flavor retainer enhancing the mouth feel, it can find applications in the preparation of emulsion type meat products (Khattab, 2009). The oil absorption capacity (OAC) of flour is also a significant functional property of the legume flour as it recovers the mouth feel (Kinsella, 1976). The oil absorption capacity of any food compound is imperative for food applications because it relies mainly on its capacity to physically deceive oil by a complex capillary attraction technique.

Foaming Capacity

Foaming properties of the flour is measured by the most frequently used indices like foam expansion (FE), foam capacity (FC) and foam stability (FS). An interfacial skin is formed when the proteins disclose to keep the air bubbles in suspension and to prevent their collapse, enhancing the formation of foam. FC of fermented horse gram flour were in the range between 6.33 to 11.00%). The overall foaming capacity of fermented horse gram flour decreases with increase in fermentation period. Nevertheless, the FC of fluidized bed dried horse gram flour was higher than forced convectional tray dried horse gram due to the retention of maximum protein content which has the ability to form a cohesive layer to prevent the air bubbles from collapse (Boye *et al.*, 2010). In general, the fermented legume flours depicted low foam stability and thus, may find less application in baked and confectionary products. This is also confirmed by the report of Obatolu *et al.* (2007) that there was a maximum difference between the foam capacities of processed yam bean (1.98%) and raw yam bean (40.2%). This difference could be attributed to the processing treatments like boiling, fermenting, roasting and malting of the legumes. However, when compared with the processed yam bean flour, the fermented horse gram flour has higher foaming capacity which can be utilized well in the preparation of meringue cakes, whipped toppings and mousses.

Conclusion

The results obtained from the study indicate that fermentation of horse gram considerably increases the nutritional value of the legume whereas the drying methods adopted significantly influences both the nutritional and functional properties of the legume flour which promotes the flour quality and shelf-life of the products prepared from the processed horse gram flour. Hence, the utilization of fermented and dehydrated horse gram and its flour not only improve the nutrient utilization and bioavailability of nutrients but also upsurge its application in therapeutic food formulations for specific health conditions.

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