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Nutritional implications and flour functionality of popped/expanded horse gram

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Abstract

Utilization of horse gram and its flour in legume composite flours and products is limited due to the presence of antinutritional components, poor functional and expansion properties. Enzymatic treatment was used to improve the expansion and functional properties of horse gram to facilitate its use as an ingredient in food processing. Xylanase-mediated depolymerization of cell wall polysaccharides of horse gram lead to the development of a new expanded/popped horse gram. Expansion process of enzyme treated horse gram resulted in increased length (5.3–6.8 mm) and higher yield of expanded grains (63–98%). The expanded horse gram had lower bulk density, higher protein digestibility and more resistant starch compared to the control raw grains. Dietary fibre content of raw and processed horse gram was in the range of 14.57–16.14%. High temperature short time (HTST) conditions used during expansion process lowered the levels of phytic acid, tannins and protease inhibitors by 46%, 61% and 92%, respectively. The flour obtained from xylanase treated and expanded horse gram had higher water (204.3 g/100 g) and oil absorption capacities (98.4 g/100 g) than unprocessed flour, which had 135.8 g/100 g and 74.6 g/100 g, respectively at ambient conditions. There was a decrease in foaming capacity and foam stability in expanded gram flour. However, emulsion stability increased significantly in the processed samples. Thus, the study indicated that nutritional value and flour functionality of horse gram could be improved by processing it into a new expanded product that can be used as an ingredient in food processing.

Keywords: Horse gram; *Macrotyloma uniflorum*; Xylanase; Popped legume; Expanded dhal; Protein digestibility; Antinutritional factors; Functional properties

1. Introduction

Horse gram (*Macrotyloma uniflorum*, previously *Dolichos biflorus*) is a minor, under-exploited legume of tropics and subtropics grown mostly under dry-land agriculture. It is an important source of protein, iron and molybdenum. It has been identified as one of the potential food sources for the future by the US National Academy of Sciences (1979). It is extensively grown in India, mainly for animal feed. The use of dry seeds of horse gram as human food is limited due to its poor cooking quality, presence of high levels of enzyme inhibitors and heamagglutinin activities (Ray, 1969). The seed is reported to be high in tannins and polyphenols compared to other legumes (Kadam & Salunkhe, 1985). Horse gram is however, consumed as sprouts in many parts of India (Ghorpade, Kadam, & Salunkhe, 1986). Poor functional properties of horse gram are major limitations to use its flour in legume composite flours. Utilization of horse gram can be maximized through an understanding of its physical and chemical components and through the implementation of diverse processing strategies to facilitate the development of economically viable alternative products. Nutritional value and consumption of horse gram could be improved by processing it into a new product or ingredient that can be used in food processing.

Thermal processing has previously been suggested to improve the texture, palatability and inactivation of heat

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labile toxic compounds and enzyme inhibitors in legumes (Ghorpade et al., 1986). Popping of legumes by subjecting them to high temperatures for a short time (HTST) has been practiced in Asia, Africa and Latin America for many years. Chickpea is the most commonly puffed legume in many countries probably, because of its ideal cell wall polysaccharide composition, starch properties and relatively high content of oil. Popped kernels of chickpea and its flour are being used extensively in food processing. Similar to popped chickpea, popped horse gram may find applications in snack, confectionary and other traditional food industries. However, unlike chickpeas, cell wall polysaccharide and starch properties of horse gram renders it difficult-to-pop legume. Modification of cell wall polysaccharides by enzymatic hydrolysis may alter the cell wall structure and may lead to the popping of horse gram. The use of popped horse gram as ingredient for food processing is dependent on its functional and antinutritional properties. To convert expanded gram into flour for use as an ingredient in food processing, research must be carried out to ascertain the functional properties of the flour as well as the sensory qualities that would render the end product acceptable to consumers.

The present study is aimed at producing expanded/popped kernels and their flours from little-known legumes and explores the possibility of using them as ingredients for food processing. Specifically the study was carried out first, to prepare a popped/expanded horse gram by enzymatic modification of cell wall components and, second to evaluate the processing effects on nutritional quality and functionality of the popped horse gram flour. This is expected to give an insight into the possible utilization of popped horse gram and its flour in different food applications.

2. Materials and methods

2.1. Materials

The horse gram seeds were purchased from a local market in Mysore, India. Care was taken to purchase all the seeds from a single batch. The seeds were then taken to the laboratory in air-tight polyethylene bags, cleaned and kept in a cool and dry place prior to use. Crude xylanase from *Aspergillus niger* obtained from M/s. Kaypeeyes Biotech Pvt. Ltd., Mysore. This enzyme preparation is free from proteases and amylases. However, it does contain small amounts of hemicellulase and cellulase activities (<1% on unit basis). Termamyl was from Novozyme, Bagsvaerd, Denmark. Protease, amyloglucosidase, porcine pepsin and pancreatin were from Sigma Chemical Co., (St. Louis, MO, USA). All other reagents were of analytical grade.

2.2. Endo-xylanase assay

The activity of crude (1,4)-β-endo-xylanase from *A. niger* was assayed according to the method of Bailey, Biely, and Poutanen (1992). The reaction mixture containing 0.9 mL of 1.0% (w/v) xylan and 0.1 mL of a suitably diluted enzyme solution was incubated in 0.01 M sodium acetate buffer, pH 5.0 for 10 min at 50 °C. The reaction was stopped by adding 1 mL of 1.0% (w/v) dinitrosalicylic acid (DNS). The amount of reducing sugar liberated was determined by DNS method using xylose (Sigma) as standard. One unit of xylanase activity was defined as the amount of enzyme that produced 1 μmol of xylose equivalent per minute.

2.3. Endo-xylanase treatment of horse gram seeds

Seeds (100 g) were treated with optimized concentration of crude xylanase (2 U/g; w/w) in 300 mL of 0.01 M sodium acetate buffer, pH 5.0. This concentration was chosen after evaluating different concentrations of enzyme in the range of 0.5–5 U/g (w/w) on the expansion properties of horse gram. The enzyme treated seeds in a closed container were incubated at 50 °C in a rotary shaker (100 rpm) for 3 h. At the end of incubation time, the buffer was decanted. The enzyme reaction was terminated by treating the grains with 300 mL of 0.1 M sodium phosphate buffer, pH 7.8 and allowed to equilibrate in the same buffer at 50 °C in a rotary shaker (100 rpm) for 3 h. After equilibration, enzyme treated grains were separated from the buffer by decanting and dried at 70 °C for 7–8 h. Control samples were also subjected to the same processing treatments without adding xylanase and designated as untreated control.

2.4. Popping of horse gram seeds

The enzyme treated grains and untreated control grains were soaked in three volumes (w/v) of distilled water for 4–5 h to attain saturation. The grains at saturated moisture content (57.2%) were popped using hot sand (1:6) at a temperature ranging from 230 to 250 °C for a short time (20–30 s) (Kurian, 1981). Expanded horse gram was separated from the sand by sieving and husks were manually removed. Dehusked kernels were dried at 70 °C for 7–8 h. Dried and expanded kernels were used for determining the physico-chemical properties and powdered as described below.

2.5. Physico-chemical properties

The percentage of expanded grains was calculated by manually separating the expanded grains from unexpanded grains. Bulk density of raw and enzyme treated grains was determined by using the method of Okezie Onuma, and Bello (1988). Length, breadth and thickness of grains were measured using vernier calipers. Expansion volume was determined by measuring the volume of the sample before and after subjecting to expansion according to the method of Chen, Shyong, and Chang (1997).

Proximate composition was determined by the Association of Official Analytical Chemists (1975) methods. Total

dietary fibre was determined by the rapid enzymatic assay (Asp, Claves, Johnson, Halmer, & Siljestrom, 1983). Resistant starch was isolated by the enzymatic method (Mangala, Malleshi, Mahadevamma, & Tharanathan, 1999). The analytical values were evaluated from the mean of three determinations for each sample.

2.6. Preparation of seed flour samples

Dehulling of raw grains was performed by abrasive grinding of sun dried grains in a versatile *dhal mill*, designed and developed at Central Food Technological Research Institute, Mysore (Kurian, 1981). Seed flours were prepared by grinding the dehulled dhal and expanded grains with a laboratory type hammer mill into fine particle size ($\leqslant$300 μm). The flour was then preserved in dry air-tight jars at 4 °C prior to use.

2.7. In vitro protein digestibility

In vitro protein digestibility of horse gram whole pulse, dehusked dhal and expanded dhals were determined by modifying the method of Akeson and Stahmann (1964). Flour sample (200 mg) was suspended in 15 mL HCl solution (0.1 M) containing 2 mg pepsin. The suspension was incubated for 3 h at 37 °C and then neutralized with 0.2 M NaOH to adjust the pH to 7.5–8.0. Phosphate buffer (7.5 mL, pH 7.4) containing 4 mg of pancreatin, 1 mM $CaCl_2$ and 0.01% NaN_3 were added to the suspension. The mixture was then further incubated for 24 h at 37 °C for digestion to occur. Enzyme blanks were prepared under the same conditions without flour samples. The undigested protein was precipitated by adding 30% trichloroacetic acid (TCA) solution and separated by ash less filter paper (Whatman No. 41). The nitrogen content of the samples and of the undigested residue was determined by Kjeldahl method. Protein digestibility was calculated using the following equation:

$$\text{Protein digestibility}(\%) = \text{Total protein in the sample} - \text{undigested protein/total protein} \times 100.$$

2.8. Antinutritional factors

The concentration of total phenolic compounds in dehusked raw and expanded horse gram dhal was measured according to the method described by Yen and Hsieh (1998). A calibration curve for tannic acid (20–100 μg) was used and the phenolic compounds were expressed as tannic acid. Phytic acid was determined according to the method described by Haug and Lantzsch (1983). The phytic acid content was calculated from a calibration curve using phytate phosphorus salt in the range of 10–50 μg. Trypsin inhibitory activity (TIA) in dehusked raw and expanded horse gram dhal was determined according to the method described by Kakade, Rackis, McGhee, and Puski (1974) with the following modifications. The inhibitor was extracted from 1 g of sample using 10 mL of 0.1 M Tris–HCl buffer (pH 8.2) at 4 °C with stirring overnight. The inhibitor concentration was optimized to obtain 40–70% inhibition of protease. This optimized concentration was used to determine the TIA. In a tube, 200 μL of filtered sample extract were mixed with 100 μL of trypsin solution (100 μg/mL in 0.02 M glycine–HCl buffer) and diluted to 1 mL with Tris–HCl buffer. A 2.5 mL volume of 1 mM benzyl-DL-argenine-*p*-nitroanilide hydrochloride (BAPNA) solution in Tris–HCl buffer (pH 8.2), previously warmed to 37 °C, was added rapidly to the tube using a micropipette and gently stirred immediately using a vortex mixer. The absorbance of each solution was measured at 410 nm against a reagent blank. A blank sample containing buffer instead of sample was prepared by the same procedure. One trypsin activity unit (TU) was defined as an increase of 0.1 absorbance unit at 410 nm. Trypsin inhibitory activity (TIU) was defined in terms of trypsin units inhibited per gram sample.

2.9. Functional properties

Flour water absorption capacity (WAC) was determined according to the method of Anderson, Conway, Pfeifer, and Griffin (1969). Flour water solubility index (WSI) was determined from the amount of dried solids recovered by evaporating the supernatant from the flour water absorption test (Anderson et al., 1969). Flour Oil absorption capacity (OAC) was estimated by centrifuging a known quantity of flour saturated with peanut oil after the procedure of Sosulski (1962). The amount of oil retained was calculated by the measurement of difference in the weight of the sample before and after equilibration with oil. Foaming properties [foaming capacity (FC) and foaming stability (FS)] were determined according to the method described by Chau and Cheung (1998). Emulsifying properties [Emulsion activity (E_a) and Emulsion stability (E_s)] were evaluated essentially according to the method of Yatsumatsu, Sawada, and Moritaka (1972).

2.10. Statistical analysis

The experimental results were subjected to the analysis of variance (ANOVA) and Duncan's multiple-range test after calculating their mean ± standard deviation (Duncan, 1955).

3. Results and discussion

3.1. Xylanase-mediated degradation of cell wall components

Plant cell walls constitute the key structural components of plants and many plant based foods. They play a central role in determining the quality characteristics from organoleptic texture to the properties of dietary fibre (Waldron, Parker, & Smith, 2003). The processing and quality

characteristics of legumes are dependent on the chemical composition of seed coat and cell wall materials. The cell wall of legume seed is a network of cellulosic microfibrils embedded in a matrix of hemicellulosic polysaccharides, protein, lipid and often phenolics (Cosgrove, 1997). Studies on various legume cell wall polysaccharide composition suggested that, the main components of hemicellulose are galactomannan, arabinoxylan and xyloglucan (Aspinall, Begbie, & McKay, 1967). Depolymerization of these polysaccharides by enzymatic hydrolysis may lead to the development of economically viable alternative products. To break-down these complex cell wall polysaccharides the enzyme preparation must have a very broad spectrum of activity. Xylanases are hydrolases, which depolymerize the components of hemicelluloses (arabinoxylan and xyloglucan). Xylanases are used in the feed industry to improve feed efficiency for poultry and other live stock (Kongbuntad, Khanongnuch, & Lumyong, 2006). Arabinoxylan degrading enzymes and various other types of enzymes have been used with excellent results to enhance oil recovery from oil seeds and fruits both on a laboratory and industrial scale (Dominguez, Nunez, & Lema, 1994). The major neutral sugars identified in the soluble and insoluble non starchy polysaccharide (NSP) fractions of horse gram were arabinose, xylose and glucose (Bravo, Siddhuraju, & Calixto, 1999). This Compositional analysis of horse gram NSP may indicate the presence of arabinoxylan and xyloglucan as constituents of hemicelluloses similar to other legumes (Aspinall et al., 1967). Therefore, xylanase was used to degrade hemicelluloses in grain cell walls of horse gram to facilitate the development of a new product or ingredient that can be used in food processing. Xylanase treated horse gram, when subjected HTST resulted in the expansion of starch in cotyledons and split the husk. Partial degradation of the components of cell wall hemicelluloses by xylanase may have facilitated the heat to pass through the cell walls and provided a space for the starch to expand under the conditions of popping.

3.2. Expansion properties of popped horse gram

The expansion properties of raw and processed horse gram are summarized in Table 1. Length and breadth of enzyme treated and expanded grains were increased by more than 45% compared to the raw dhal. The thickness of untreated grains (control) upon HTST treatment increased from 1.82 to 2.04 mm whereas, it remained the same in enzyme treated expanded grain (1.8 mm). Increased thickness of untreated expanded grains may be due to the incomplete expansion of starch. About 98% of the grains were expanded in the enzyme treated sample compared to 63% in untreated control without enzyme. Similar to chickpea, a traditionally popped legume, the expansion volume of the xylanase treated and popped horse gram reached 1.5 times (1.1 times in control) differing significantly ($P < 0.05$). The puffing expansion of grain legumes is generally low (1.2–1.5 times) compared to cereals (8–10 times). The popping process decreased the bulk density by 40%. On application of statistical test, it was evident that bulk densities of expanded horse gram were significantly different from raw dhal at the 5% level of probability. It may be expected that decreased bulk density of expanded horse gram may find application in the preparation of weaning food formulations.

3.3. Seed composition

Proximate composition of dehusked raw dhal and expanded dhal is presented in Table 2. The protein content of horse gram dhal is comparable to other legumes. How-

Table 1
Expansion properties of raw and expanded horse gram

Parameter	Raw dehusked dhal	Expanded dhal (untreated control)	Xylanase-treated and expanded dhal
Length (mm)[A]	4.60 ± 0.08^{c}	5.30 ± 0.05^{b}	6.80 ± 0.03^{a}
Breadth (mm)[A]	3.10 ± 0.04^{b}	3.45 ± 0.08^{b}	4.50 ± 0.10^{a}
Thickness (mm)[A]	1.82 ± 0.09^{b}	2.04 ± 0.06^{a}	1.80 ± 0.05^{b}
Grains expanded (%)[B]	00.00	63.20 ± 2.7^{b}	98.40 ± 3.2^{a}
Bulk density (g/mL)[B]	0.83 ± 0.02^{a}	0.68 ± 0.04^{b}	0.50 ± 0.02^{b}
Expansion volume (%)[B]	00.00	116 ± 5.1^{b}	155 ± 4.6^{a}

Mean values bearing different letters a, b, c in the same row are significantly different ($P < 0.05$) on application of Duncan's multiple range test.
[A] Results are mean ± SD of 100 grains.
[B] These results are mean ± SD of four determinations.

Table 2
Mean proximate composition and selected carbohydrate components of horse gram dhal flours, calculated as (%) of dry matter

Flour sample	Moisture	Ash	Fat	Protein	Carbohydrate[A]	Total dietary fiber	Resistant starch
Dehusked dhal (raw)	8.83^{a}	2.72^{a}	1.25^{a}	22.50^{b}	64.70^{a}	15.08^{a}	1.63^{b}
Expanded dhal (untreated control)	8.26^{a}	1.87^{b}	1.30^{a}	21.65^{b}	66.92^{a}	16.14^{a}	2.08^{a}
Xylanase-treated and expanded dhal	8.8^{a}	1.65^{b}	1.12^{a}	25.70^{a}	62.72^{a}	14.57^{a}	1.72^{b}

Values are mean ± standard deviation of three independent determinations. Means with the same superscript (a, b) within the same column do not differ ($P > 0.05$).
[A] By difference as 100 − (moisture + protein + ash + fat).

ever, xylanase-treated expanded dhal had higher protein compared to dehusked raw dhal and untreated expanded dhal differing statistically ($P < 0.05$). This was counteracted by a lower content of carbohydrates. Degradation of some of the cell wall non starchy polysaccharides by xylanase may have resulted in a lower carbohydrate and a higher protein content in the enzyme treated grain. Carbohydrate, determined by difference, presented similar statistical values, accounting for more than 60% of the grain composition. Ash and fat content of raw and processed horse gram was similar to other more commonly consumed legumes (Bravo et al., 1999).

Raw and processed horse gram contain similar content of the total dietary fibre in the range of 14.57–16.14% (Table 2). However, statistically significant higher resistant starch value (2.08%) was observed for the untreated expanded grain. Compared to the control, the enzyme treated grain had lower RS content (1.72%). The observed lower RS content in enzyme treated grain may be due to the hydrolysis of cell wall non starchy polysaccharides by xylanase resulting in more access and rapid hydrolysis of inaccessible starch embedded in the insoluble and soluble fractions of NSP by amylase.

3.4. In vitro protein digestibility (IVPD)

Legumes are known to have a lower protein digestibility, which is attributed to the presence of antinutritional factors (Liener, 1994). Horse gram contains several natural constituents such as protease inhibitors, haemaglutinins, tannins and phytic acid, which can interfere with protein digestion or disrupt physiological processes. *In vitro* protein digestibilities of horse gram whole pulse, dehusked dhal and expanded dhal are shown in Fig. 1. Horse gram with husk has a protein digestibility of 32.6% whereas, dehusking has improved the protein digestibility to 44.1%. Rehman, and Salariya (2005) reported protein digestibility of various raw legumes in the range of 33.8–37.6%. Very low protein digestibility of raw horse gram could be due to the presence of high amounts of trypsin inhibitors and tannins compared to other legumes. Since, most of the seed tannins are present in the seed coat, removal of seed coat by dehulling increased the digestibility by 35% (Fig. 1). Processing affected the IVPD of flour. Untreated expanded horse gram flour has a digestibility value of 68.3%, whereas, enzyme treatment increased the IVPD to 76.8%. Therefore, on the basis of these results, it was concluded that degradation of cell wall hemicelluloses by xylanase resulted in increased IVPD (74.15%) compared to the untreated control (54%).

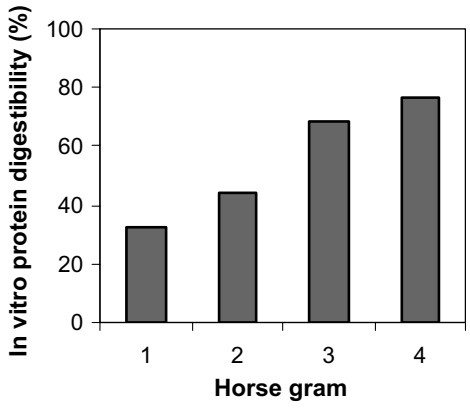

Fig. 1. *In vitro* protein digestibility of raw and processed horse gram. (1) Horse gram whole pulse, (2) dehusked raw dhal, (3) expanded dhal (untreated control) and (4) Xylanase treated and expanded dhal. Results are means of triplicate determinations.

The IVPD of processed horse gram compared favorably with the reported IVPD of other food legumes (Rehman & Salariya, 2005). The higher protein digestibility after heat treatment may be due to increased accessibility of protein to enzymatic attack. However, this effect could also be due to the inactivation of protease inhibitors. Our results are consistent with the findings of Van der Poel, Gravandecl, and Boer (1991), who reported a 76–82% improvement in the protein digestibility of faba beans as a result of thermal processing.

3.5. Antinutritional factors

The contents of antinutritional factors of raw and expanded horse gram are shown in Table 3. The presence of antinutritional factors is one of the major drawbacks limiting the nutritional and food qualities of legumes (Liener, 1994). Dehusked raw horse gram dhal contain 7.48 mg/g phytic acid. Comparing the phytic acid contents of other common food legumes, it is lower than the values reported for chickpea (9.7 mg/g), black gram (11 mg/g), lentil (12.5 mg/g), red kidney bean (14.4 mg/g) and white kidney bean (12.3 mg/g) (Rehman & Salariya, 2005). However, the phytic acid content in horse gram is higher than the levels reported for pigeon pea (2.2 mg/g) and bambara groundnut (2.9 mg/100 g) (Igbedioh, Olugbemi, & Akpapunam, 1994). Besides lowering the bioavailability of minerals and inhibiting the digestibility of proteins, phytic acid is also implicated in the "hard-to-cook" phenomenon of legumes (Stanley & Aguilera, 1985). Thermal treatment (popping) significantly ($P < 0.5$) reduced phytate content of horse gram to 4.02 mg/g in untreated control and 3.87 mg/g in xylanase treated expanded dhal. These results are consistent with reduction of phytic acid in various other legumes and oil seed flours by thermal treatment. Various processing methods such as germination, boiling, fermentation and roasting reduced the phytate content in cashew nut, fluted pumpkin and bread nut seed flours (Fagbemi, Oshodi, & Ipinmoroti, 2005). Autoclaving and roasting were more effective in reducing phytic acid in chickpea and pigeon pea than black gram, mung bean and soybean (Chitra, Singh, & Rao, 1996).

The polyphenol content of dehusked raw horse gram dhal showed in Table 3 (0.93%) is high than those reported for many other common food legumes (Ghorpade et al., 1986). Phenolic compounds usually form insoluble

Table 3
Antinutritional factors in raw and expanded horse gram[A]

Antinutrional factor	Raw seeds (dehusked dhal)	Expanded dhal (untreated control)	Xylanase-treated and expanded dhal
Phytic acid (mg/g)	7.48 ± 0.5^{a}	4.02 ± 0.1^{b}	3.87 ± 0.1^{b}
Poly phenols (mg TA/g)	9.31 ± 0.3^{a}	3.59 ± 0.05^{b}	3.35 ± 0.2^{b}
Trypsin inhibitor activity (units/g)	$10{,}200 \pm 15.2^{a}$	846 ± 3.4^{b}	761 ± 4.5^{c}

Mean values bearing different letters a, b, c in the same row are significantly different ($P < 0.05$) on application of Duncan's multiple range test.
[A] Results are mean ± standard deviation of triplicate determinations.

complexes with protein, thereby interfering with their bioavailability (Liener, 1994). Expansion of horse gram resulted in a significant ($P < 0.5$) reduction of phenolic compounds by 61% in untreated control and 64% in enzyme treated expanded dhal. Boiling and popping of amaranth seeds resulted in reduction of phenolic compounds by 51.5% and 19.1%, respectively (Gamel, Linssen, Mesallam, Damir, & Shekib, 2006). In our experiments with horse gram, popping method is more effective in elimination of phenolic compounds. This effect was most probably related to the high temperature (230–250 °C) used in popping. These results are in good agreement with those of Fagbemi et al. (2005), who found that roasted bread nut, cashew nut and fluted pumpkin flours had 36.7%, 36.1% and 48.2% lower tannins than the unprocessed and raw seed flours.

Trypsin inhibitory activity (TIA) of raw and expanded horse gram is shown in Table 3. Though the trypsin inhibitory activity has been studied in a number of legumes, the results obtained in the present investigation cannot be compared with them because the expression of trypsin inhibitor activity, nature, and concentration of the substrates, etc., are different. However, based on the investigations carried out by Chau, Cheung, and Wong (1997) under similar experimental conditions for trypsin inhibitory activity in food legumes like cowpea (2240 TIA), field bean (3400 TIA) and *Phaseolus calcaratus* (2280 TIA), it may be inferred that the TIA obtained in the present study (10200 TIA) appears to be very high. Previously, we have purified and characterized a heat stable, double-headed trypsin/chymotrypsin inhibitor (Bowman–Birk type) from horse gram (Sreerama & Gowda, 1997). Thermal stability studies of this inhibitor indicated its remarkable stability to dry heat at 80 °C for 10 min. The stability of the inhibitor was attributed to seven disulphide bridges within the small molecular size (MW 8000 Da) polypeptide. In the present investigation, high moisture of the grain and higher temperature used for popping resulted in the loss of more than 90% TIA. Amaranth seeds popped at 180 °C lost 86–88% TIA (Gamel et al., 2006). Similarly, roasting of bread nut seed, cashew nut and fluted pumpkin flours resulted in 26.8%, 58.7% and 100% reduction in TIA, respectively (Fagbemi et al., 2005). Trypsin inhibitors ingested in significant amounts disrupt the digestive process and may lead to undesirable physiological reactions. Thus, the results obtained from this study showed that the popping of horse gram increased the nutrient availability by significantly reducing the contents of antinutrional factors such as phytic acid, tannins and trypsin inhibitors. Expanded horse gram kernel or flour could be used as ingredient for food processing to improve the bioavailability of nutrients in complex food system.

3.6. Functional properties

Studies on functional properties are important for the efficient utilization and consumer acceptance of raw and expanded horse gram flour.

3.6.1. Water absorption capacity (WAC) and water solubility index (WSI)

The enhanced ability of flour to absorb and retain water and oil may help to improve binding of the structure, enhance flavour retention, improve mouthfeel and reduce moisture and fat losses of food products. WAC of raw dehusked horse gram flour was 135.8 g/100 g (Table 4). This is comparable to reported values for green gram (122.6 g/100 g), cowpea (128.5 g/100 g), and chickpea (136.2 g/

Table 4
Functional properties of flours obtained from dehusked raw and expanded horse gram[A]

Functional property	Dehusked raw dhal flour	Expanded dhal flour (untreated control)	Xylanase-treated and expanded dhal flour
Water absorption capacity (g/100 g)	135.8 ± 3.8^{c}	174.2 ± 3.6^{b}	204.3 ± 3.6^{a}
Water solubility index (%)	7.6 ± 0.5^{b}	14.8 ± 0.6^{a}	18.4 ± 0.4^{a}
Oil absorption capacity (g/100 g)	74.6 ± 1.8^{c}	81.6 ± 2.8^{b}	98.4 ± 1.7^{a}
Foaming capacity (%)	45.0 ± 1.8^{a}	38.4 ± 1.5^{b}	25.0 ± 0.6^{c}
Foam stability (%)	38.0 ± 1.5^{a}	21.6 ± 0.8^{b}	12.0 ± 0.3^{c}
Emulsion activity (%)	52.6 ± 1.8^{a}	59.0 ± 2.2^{a}	64.1 ± 1.3^{a}
Emulsion stability (%)	48.2 ± 0.9^{c}	52.1 ± 1.9^{b}	59.7 ± 1.5^{a}

Mean values bearing different letters a, b, c in the same row for each functional property are significantly different ($P < 0.05$) on application of Duncan's multiple range test.
[A] Values are mean ± standard deviation of three independent determinations.

100 g) (Ghavidel & Prakash, 2006). However, WAC values of horse gram flour was higher than the reported WAC values for lentil (97.4 g/100 g) and other varieties of horse gram (92–114 mL/100 g) (Diwakar, Kushwah, & Kushwah, 1996; Ghavidel & Prakash, 2006), whereas, amaranth flour (180–230 g/100 g) and dry red bean flour (205.1 g/100 g) had higher WAC values compared to horse gram (Gamel et al., 2006; Njintang, Mbofung, & Waldron, 2001). It is known that polar amino acid residues of proteins have an affinity for water molecules (Kinsella, 1976) and differences in WAC of different legumes could be due to the content of these amino acids in legumes. In addition to proteins, polysaccharides also affect water absorption. Popping process significantly ($P < 0.05$) increased WAC of horse gram flour from 135.8 g/100 g in raw flour to 174.2 g/100 g expanded dhal. This increase is mainly due to starch gelatinization and protein denaturation. Xylanase treatment further increased the WAC of expanded dhal flour to 204.3 g/100 g. (Table 4). Partial hydrolysis of cell wall components by xylanase may have facilitated the heat to pass through the cell walls resulting in complete gelatinization of starch and denaturation proteins. Popped *Amaranthus caudatus* and *Amaranthus cruentus* seed flours, had 122% and 250% increased water holding capacities, respectively compared to control. (Gamel et al., 2006). The water solubility index (WSI) values of untreated control and enzyme treated expanded horse gram flour were 95% and 142% higher than that of the raw flour (Table 4).

3.6.2. Oil absorption capacity (OAC)

The oil absorption capacity of raw dehusked horse gram flour was 74.6 g/100 g (Table 4), which is similar to those reported for chick pea (78.8 g/100 g) (Ghavidel & Prakash, 2006). Popping process significantly enhanced the OAC of popped horse gram flour to 81.6 g/100 g in untreated control and 98.4 g/100 g enzyme treated expanded flour. The mechanism of oil absorption may be explained as a physical entrapment of oil related to the non polar side chains of proteins. Nature of primary structure and conformational features, hydrophobic amino acid concentrations and protein content all contribute to the oil-retaining properties of food materials. Any processing method that influences these parameters would tend to influence the oil absorption characteristics of the food system (Njintang et al., 2001). The micro-porous nature of the expanded products may also expose more proteins to the surface to interact with oil. The observed effect of expansion suggests that all these factors may have influenced the nature of proteins in horse gram. The popped horse gram flour may be useful in ground meat formulations, meat replacers and extenders, pancakes, backed goods and soups, where oil holding capacity is of prime importance.

3.6.3. Foaming properties [foaming capacity (FC) and foaming stability (FS)]

Owing to a large increase in the surface area in the liquid/air interphase, proteins denature and aggregate during whipping. This is important for flour used in many leavening food products such as baked goods, cakes and biscuits. Table 4 show the foaming capacity and foaming stability of raw and popped horse gram flours. Raw horse gram had 45% FC and 38% FS. Popping process of horse gram resulted in a significant decrease of FC and FS. Significantly higher decreases in FC and FS values were noted for enzyme treated expanded flour (25% and 12%, respectively), compared to untreated expanded dhal flour (FC:38.4% and FS:21.6%). The reduction was related to protein denaturation. These results agree with the finding of Lin, Humbert, and Sosulski (1974) that the native protein gives higher foam stability than denatured protein. Thermal treatment caused a significant reduction in the foaming capacity and foaming stability of amaranth flour (Gamel et al., 2006).

3.6.4. Emulsifying properties (emulsion activity (E_a) and emulsion stability (E_s)

Raw horse gram flour had E_a and E_s of 52.6% and 48.2%, respectively. These values are comparable to the reported E_a values for green gram, cowpea, lentil and chickpea (48–54%) (Ghavidel & Prakash, 2006). However, popped horse gram flour had higher E_a and E_s values (Table 4). The enzyme treated and expanded dhal flour had E_a and E_s values of 64.1% and 59.7%, respectively, whereas untreated control sample had marginally lower E_a and E_s values (59.0% and 52.1%, respectively). The increased E_a may be due to the protein denaturation resulting in more exposed hydrophobic amino acid residues. E_s denotes the ability of an emulsion with a certain composition remain unchanged. E_s is an important functional property of the flour, which helps in fat/water phase stability in many baked food products.

4. Conclusions

Modification of cell wall polysaccharides of horse gram by enzymatic hydrolysis may have altered the cell wall structure leading to the development of expanded horse gram. Popping/expansion of horse gram improved the *in vitro* protein digestibility and reduced the levels of antinutritional factors such as phytate, tannins and trypsin inhibitor. The processing of horse gram also influenced the functional properties of the flour. Higher WAC and OAC properties of popped horse gram flour may find application as fat and water binder in meat products such as sausages and canned meat. The significant changes induced by popping, on proteins and starch structure modified the water holding capacity and emulsifying properties of puffed horse gram flour. These changes suggest that flour from puffed gram could be used not only as ingredient in snack formulations, but also as a means to control water migration in baked products and soups. In the face of growing urbanization in developing countries, popped or expanded horse gram with reduced antinutritional factors and improved functional properties may satisfy the nutri-

tional needs of consumers. This study also demonstrated that use of enzymes has the potential to increase efficiency and quality output in grain processing operations in many emerging countries.

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