



Nutrients and antinutrients in cowpea and horse gram flours in comparison to chickpea flour: Evaluation of their flour functionality

Yadahally N. Sreerama^{*}, Vadakkoot B. Sashikala, Vishwas M. Pratape, Vasudeva Singh

Department of Grain Science and Technology, Central Food Technological Research Institute, Council of Scientific and Industrial Research (CSIR), Mysore 570020, India

ARTICLE INFO

Article history:

Received 24 March 2011

Received in revised form 15 July 2011

Accepted 6 September 2011

Available online 12 September 2011

Keywords:

Underutilized legumes

Nutrients

Antinutritional factors

Flour functionality

Composite flour

Legume flour

ABSTRACT

The nutrient composition, antinutritional factors and flour functionality of cowpea and horse gram flours were evaluated in comparison to chickpea flour characteristics. Protein content of these flours was in the range of 22.5–24.1%. Fat content was highest in chickpea (4.8%) and lowest in horse gram (1.4%). Resistant starch and phytic acid were significantly higher in cowpea flour. Compared to chickpea and cowpea, horse gram has more carbohydrate, dietary fibre, polyphenols and trypsin inhibitor activity and less oligosaccharides. Horse gram and chickpea flours had the highest water and oil absorption capacities with values of 148.1% and 109.3%, respectively. Although emulsion activity (58.1%) and stability (52.0%) were superior in horse gram, foaming capacity and foam stability were significantly higher in chickpea and cowpea flours, respectively. These results suggest the potential utility of cowpea and horse gram flours as substitutes for chickpea flour in some food products formulations.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

As good sources of proteins, carbohydrates, several water-soluble vitamins, and minerals, legumes in general make a major contribution to human nutrition. Chickpea (*Cicer arietinum* L.) is one of the oldest and most widely consumed legumes in the world, particularly in tropical and subtropical areas. Chickpea and its flour (garbanzo flour or besan) are being used extensively in food processing in many countries because of its ideal cell wall polysaccharide composition, versatile flour functionality and relatively high content of oil. It is a staple ingredient in south and southeast Asian cuisines. Its flour is the main ingredient in many Indian sweets, desserts and savoury products. It is also used in Italian and French cuisines to make variety of desserts, noodles, snacks and main dishes (Alajaji & El-Adawy, 2006). However, other underutilised legumes, such as cowpea (*Vigna unguiculata* L. Walp.) and horse gram (*Macrotyloma uniflorum* L.) have been recognised as potential sources of protein and other nutrients (Prinyawiwatkul, McWatters, Beuchat, & Phillips, 1996; National Academy of Sciences, 1979). Cowpea is cultivated for its immature pods and mature seeds and is consumed extensively in Africa and, to a lesser extent, in Asia (Prinyawiwatkul et al., 1996). Similarly, horse gram is largely cultivated, especially in dry areas of Australia, Burma, India and Sri Lanka, mainly for animal feed. It is also used as a vegetable in India and is known as the poor man's pulse crop in southern India (Kadam & Salunkhe, 1985). Both

cowpea and horse gram are low in fat and are excellent sources of protein, dietary fibre, a variety of micronutrients and phytochemicals (Kadam & Salunkhe, 1985; Siddhuraju & Becker, 2007).

The use of cowpea and horse gram flours, as ingredients in composite flours and functional foods, is limited, due to the presence of certain phytochemicals with antinutrient effects that limit the nutritive value of these legumes. Conventional processing methods, such as soaking, boiling, germination, and fermentation, are widely used to decrease the content of these undesirable components, which results in enhanced acceptability and nutritional quality in addition to optimal utilisation of these legumes as human food (Kadam & Salunkhe, 1985). However, recently, health-promoting and disease-preventing properties have been attributed to these phytochemicals with antinutrient effects, thus attracting more and more interest from both researchers and food manufacturers (Jacobs & Steffen, 2003). Improved utilisation of these underutilised legume flours can be maximised through an understanding of their physical and chemical components and through the implementation of diverse processing strategies to facilitate the development of economically viable alternative products. In this respect, some recent studies have indicated that the consumption of cowpea and horse gram could be improved by processing them into ingredients that can be used in food product applications (Prinyawiwatkul et al., 1996; Sreerama, Sashikala, & Pratape, 2008).

The present study is aimed at producing composite flours from little-known legumes, such as cowpea and horse gram, and exploring the possibility of using them as ingredients for food processing. However, the use of legume flours in various food formulations is

^{*} Corresponding author. Tel.: +91 821 2510843; fax: +91 821 2517233.

E-mail address: yans@cftri.res.in (Y.N. Sreerama).

dependent on their nutritional and functional properties. Therefore, an investigation was carried out first, to elucidate the nutritional and functional properties of cowpea and horse gram flours in comparison to chickpea flour characteristics. This is expected to give insight into the possible utilisation of cowpea and/or horse gram flour as partial substitutes of chickpea flour in snack, confectionary and other traditional food products.

2. Materials and methods

2.1. Chemicals

The Folin–Ciocalteu reagent, gallic acid, bovine trypsin, benzyl-DL-arginine-*p*-nitroanilide hydrochloride (BAPNA), raffinose, stachyose and verbascose were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade.

2.2. Sample preparation

Cowpea, horse gram and chickpea seeds commonly cultivated in India were purchased from a local market in Mysore, India. Dehulling and separation of hulls from cotyledons were done as described previously (Sreerama, Neelam, Sashikala, & Pratape, 2010), using a grain-testing device (Strong-Scott Ltd., Winnipeg, MB, Canada). The separated, dehulled cotyledons were ground using a coffee bean grinder, to obtain a fine powder that passed through a 60 mesh sieve. All samples were defatted by blending with hexane (1:5 w/v, 5 min, three times) in a Waring blender at ambient temperature and air-dried for 12 h. Defatted flours were vacuum-packed in polythene pouches and stored in the dark, at 4 °C, until used.

2.3. Nutrient composition

Analyses of cowpea, horse gram and chickpea flours for crude protein, fat, ash and moisture contents were carried out essentially according to the standard methods of the Association of Official Analytical Chemists (1990). The carbohydrate content was determined as the weight difference using moisture, crude protein, lipid and ash content data. Total dietary fibre (TDF) was determined by rapid enzymatic assay (Asp, Claves, Johnson, Halmer, & Siljestrom, 1983). Resistant starch was isolated and determined by an enzymatic method (Mangala, Malleshi, Mahadevamma, & Tharanathan, 1999). The analytical values were evaluated from the mean of three determinations for each sample.

2.4. Antinutritional factors

2.4.1. Phenolic compounds

Total phenolics from defatted legume flours were extracted with 80% aqueous methanol containing 1% HCl (1:50 w/v) by refluxing in a boiling water bath for 30 min ($\times 3$ times). The refluxed material was concentrated under vacuum in a rotary flash evaporator and the total phenolic content was measured according to the method of Singleton, Orthofer, and Lamuela-Raventos (1999). The content of total phenolics in each extract was determined, using a standard curve prepared for gallic acid, and expressed as mg of gallic acid equivalents (GAE) per gramme of defatted meal flour.

2.4.2. Phytate content

Phytic acid contents of defatted legume flours were determined by the method of 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 .

2.4.3. Trypsin inhibitory activity (TIA)

Trypsin inhibitor was extracted from 1 g of flour, using 10 ml of 0.1 M Tris–HCl buffer (pH 8.2) at 4 °C with stirring overnight. TIA of flours was determined according to the method of Kakade, Rackis, McGhee, and Puski (1974) with modifications, as described by Sreerama, Sashikala, & Pratape (2008). The inhibitor concentration was optimised to obtain 40–70% inhibition of trypsin. One trypsin activity unit was defined as an increase of 0.1 absorbance unit at 410 nm. Trypsin inhibitory activity has been defined in terms of trypsin units inhibited per gramme of sample. Appropriate controls, containing buffer instead of sample, were maintained. Inhibition of trypsin activity was also determined in the presence of various concentrations of buffer extracts of legume flours (15–75 $\mu\text{g}/\text{ml}$) to derive the IC_{50} values. IC_{50} was defined as the concentration of extract required to inhibit 50% of the enzyme activity and obtained graphically using an inhibition curve.

2.4.4. Flatulence factors

Flatulence-forming oligosaccharides were first extracted from defatted cowpea, horse gram and chickpea flours by treating 5 g of each sample with 25 ml of 80% ethanol at room temperature (27 ± 2 °C) and stirring on a magnetic stirrer for 30 min. The extraction was repeated thrice. The extracts were pooled and concentrated using a rotary evaporator under vacuum. The residue was made up to 5 ml with deionised water and the sugars were separated on a Kromasil NH_2 analytical HPLC column (250×4 mm, particle size 5 μm ; Phenomenex Torrance, CA, USA) according to the method of Sreerama et al. (2010), using a Shimadzu HPLC system (LC-10ATVP, Shimadzu Corporation, Japan). Data signals were acquired and processed on a PC running the Class VP software. Oligosaccharides in the extract were detected using a refractive index detector (RID-10A) and identified by comparing their retention times with those of known standards. Standards used were raffinose, stachyose and verbascose.

2.5. Functional properties

2.5.1. General

The functional properties of non-defatted flours were evaluated under the same conditions according to the following methods.

2.5.2. Nitrogen solubility

The soluble nitrogen contents of legume flours, as a function of pH, were determined by extraction of the protein at different pH values ranging from 2 to 10 according to the method described by Boye et al., 2010. The amount of protein in the supernatant was determined by the method of Bradford (1976). The percentage of soluble protein was calculated as the percentage ratio of protein in the supernatant to that of the total protein in the initial sample.

2.5.3. Water and oil absorption capacity

Flour water absorption capacity (WAC) was determined according to the method described by 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 measurement of difference in the weights of the sample before and after equilibration with oil.

2.5.4. Foaming and emulsifying properties

Foaming capacity (FC) and foaming stability (FS) were determined according to the method described by Chau and Cheung (1998). Emulsion activity (Ea) and emulsion stability (Es) were

evaluated essentially according to the method of Yatsumatsu, Sawada, and Moritaka (1972).

2.6. Statistical analysis

All measurements were carried out in triplicate. Data obtained were analysed using a one-way analysis of variance (ANOVA) with significance at $p < 0.05$. Significant differences among mean values were determined by Duncan's multiple range test (Duncan, 1955).

3. Results and discussion

3.1. Nutrient composition

Nutrient composition of chickpea, cowpea and horse gram flours is shown in Table 1. The moisture content of chickpea flour was significantly higher than those of cowpea and horse gram flours. Protein content of the three flours was in the range of 22.5–24.1%, with no statistically significant difference between the flours. The protein content is comparable to those of other edible leguminous seed flours, such as pigeon peas, some varieties of cowpea and soya beans (Olaofe, Adeyemi, & Adediran, 1994). Total carbohydrate, determined by difference, accounted for more than 60% of the grain composition. There was no significant difference between the carbohydrate content of chickpea and cowpea flours. However, horse gram flour has more carbohydrates. The fat content of the three flours ranged from 1.4% to 4.8% with statistically significant differences between the flours. Horse gram flour had the lowest value of fat while chickpea had the highest value. More fat in chickpea flour might be disadvantageous in terms of the shelf life and keeping qualities of this flour. However, this is an important property of chickpea flour, enabling its wide utilisation in food products. Higher fat in chickpea flour also enhances the ability of flour to absorb and retain oil, improves binding of the structure, enhances flavour retention, improves mouth feel and reduces moisture and fat losses of food products (Sreerama, Sashikala, & Pratape, 2008). The lower lipid content of horse gram flour may be utilised as ingredients in weight restriction diets. Compared with cowpea and horse gram, chickpea flour recorded the lowest ash content.

Horse gram flour contains the highest amount of TDF, followed by chickpea and cowpea flours. Similarly, the insoluble dietary fibre (IDF), which comprises lignin, cellulose and some hemicelluloses and soluble dietary fibre (SDF) were also higher in horse gram flour (Table 1). Among the flours, cowpea contains the lowest amount of IDF. These values are similar to the values reported for chickpea (Costa, Queiroz-Monici, Reis, & Oliveira, 2006) and horse gram (Sreerama, Sashikala, & Pratape, 2008). The high content of dietary fibre in horse gram flours might be helpful in terms of maintaining positive effects on intestine and colon physiology,

Table 1
Nutrient composition of legume flours, calculated as % of dry matter.*

Constituent	Chickpea	Cowpea	Horse gram
Moisture	8.2 ± 0.4 ^a	7.4 ± 0.2 ^b	6.8 ± 0.2 ^b
Protein	23.7 ± 1.1 ^a	24.1 ± 0.9 ^a	22.5 ± 1.0 ^a
Carbohydrate [#]	61.1 ± 1.8 ^b	63.3 ± 1.2 ^b	66.6 ± 2.1 ^a
Fat	4.8 ± 0.1 ^a	2.3 ± 0.0 ^b	1.4 ± 0.0 ^c
Ash	2.2 ± 0.0 ^b	2.9 ± 0.0 ^a	2.7 ± 0.0 ^a
Total dietary fibre	14.8 ± 0.4 ^b	14.1 ± 0.3 ^b	16.3 ± 0.5 ^a
Soluble	1.1 ± 0.0 ^b	1.0 ± 0.0 ^b	1.4 ± 0.0 ^a
Insoluble	13.7 ± 0.4 ^b	13.1 ± 0.2 ^c	14.9 ± 0.4 ^a
Resistant starch	1.9 ± 0.2 ^b	2.5 ± 0.1 ^a	2.2 ± 0.2 ^b

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.

* Results are means ± standard deviation of triplicate determinations.

[#] By difference as 100-(moisture + protein + ash + fat).

besides other homeostatic and therapeutic functions in human nutrition. The results presented in Table 1 also indicate that legume flours contain significant quantities of resistant starch (RS). Statistically significant higher resistant starch content was observed in cowpea flour. The RS in legume flours present various physiological effects in the gastrointestinal tract of humans. These include alteration of the gastrointestinal transit time, satiety changes, influence on the levels of body cholesterol, after-meal serum glucose and insulin levels, flatulence and alteration in nutrient bioavailability (Hopwell, Yeater, & Ullrich, 1993).

3.2. Antinutritional factors

The concentrations of the antinutritional factors in flours of chickpea, cowpea and horse gram are shown in Table 2. Cowpea flour showed the highest phytic acid content, followed by chickpea and horse gram flours. These values are comparable to 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 contents of all three legumes were 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). However, its presence is also beneficial because it may have a positive nutritional role as an antioxidant and anticancer agent (Turner, Paphazy, Haygarth, & Mckelvie, 2002).

Among the different flours, the contents of total polyphenols were significantly higher in horse gram than in cowpea, which is higher than chickpea (Table 2). The phenolic content appeared to be similar to those of earlier reports in chickpea and horse gram milled fractions (Sreerama et al., 2010) and cowpea varieties (Preet & Punia, 2000). However, the phenolic content in these flours is lower than those reported for beach pea (Shahidi, Chavan, Naczka, & Amarowicz, 2001), cowpea, pea, pigeon pea and chickpea (Reddy, Pierson, Sathe, & Salunkhe, 1985). Phenolic compounds are known to interact with proteins, forming complexes which, in turn, decrease the solubility of proteins and make protein complexes less susceptible to proteolytic attack than are the same proteins alone (Reddy et al., 1985). Besides, they impair starch and disaccharide assimilation and interact with proteolytic enzymes inhibiting their activity. However, plant phenolics are receiving growing interest due to their potential role as protective factors against free radical-mediated pathologies, such as cancer and atherosclerosis in humans (Kehrer, 1993).

The contents of oligosaccharides in the three legume flours are shown in Table 2. The oligosaccharides, namely, raffinose, stachyose and verbascose, were detected in all the samples. Chickpea

Table 2
Antinutritional factors in legume flours.*

Antinutritional factor	Chickpea	Cowpea	Horse gram
Phytic acid (mg/g)	12.1 ± 0.5 ^b	14.0 ± 0.7 ^a	10.2 ± 0.4 ^c
Polyphenols (mg GA/g)	10.8 ± 0.1 ^c	12.1 ± 0.3 ^b	14.3 ± 0.4 ^a
Oligosaccharides (mg/g)	34.9	31.7	26.8
Raffinose	8.6 ± 0.0 ^b	10.3 ± 0.0 ^a	7.1 ± 0.0 ^c
Stachyose	19.1 ± 0.2 ^a	17.8 ± 0.2 ^b	15.6 ± 0.4 ^c
Verbascose	7.2 ± 0.0 ^a	3.6 ± 0.0 ^c	4.1 ± 0.0 ^b
<i>Trypsin inhibitor activity</i>			
Units/g	6452 ± 16 ^c	6981 ± 24 ^b	9246 ± 18 ^a
IC ₅₀ (µg/ml)	44.8	38.2	29.4

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.

* Results are means ± standard deviation of triplicate determinations.

flour contained the highest amount of total α -galactosides, followed by cowpea and horse gram flours. Within the oligosaccharides, stachyose was the principal oligosaccharide in all three legume flours, whereas verbascose was present in minor quantities in cowpea and horse gram flours. The contents of these oligosaccharide are comparable to the most recently reported values in the milled fractions of chickpea and horse gram (Sreerama et al., 2010). Raffinose, stachyose and verbascose α -galactosides from pulses are causative agents of flatulence in humans. These oligosaccharides are not digested by humans because the intestinal mucosa lacks the hydrolytic enzyme, α -1, 6-galactosidase. Microflora in the lower intestinal tract metabolise these oligosaccharides and produce large amounts of carbon dioxide and hydrogen and a small quantity of methane, which causes flatus production (Rackis, 1974). Accumulation of flatus in the intestinal tract results in discomfort, abdominal rumblings, cramps, pain and diarrhoea. However, it is well known that balance of intestinal bacterial flora is important, which could dominate pathogenic organisms and thus improve human health. These galacto-oligosaccharides facilitate the growth of intestinal bifidobacteria. In fact, many prebiotic oligosaccharides are used as ingredients in various products, such as soft drinks, cookies, cereals, candies and infant foods (Nakakuki, 2003). The chickpea, cowpea and horse gram flours, which contain substantial amounts of oligosaccharides, may be used as ingredients in functional foods.

The mean values for the trypsin inhibitor activities of the different legume flours are presented in Table 2. Trypsin inhibitory activity of horse gram flour was significantly higher than those of the other two flours. The TIA of cowpea flour was higher than that of chickpea flour. At the same concentration, horse gram extract inhibited trypsin to a greater extent than did chickpea and cowpea extracts (Fig. 1). The concentrations necessary to inhibit 50% of the initial trypsin activity (IC_{50}) were 44.8, 38.2 and 29.4 μ g/ml for chickpea, cowpea and horse gram, respectively (Table 2). These inhibitory activities are comparable to the reported inhibitory activities in the milled fractions of chickpea and horse gram (Sreerama et al., 2010) and cowpea flour (Prinyawiwatkul, Eitenmiller, Beuchat, McWatters, & Phillips, 1996). Protease inhibitors form stable complexes with digestive enzymes and inhibit their activity. The presence of protease inhibitors in food decreases the apparent nutritional quality of proteins in the diet by affecting the ability of body digestive enzymes to degrade dietary protein, and thus limiting the intake of amino acids needed to construct new proteins.

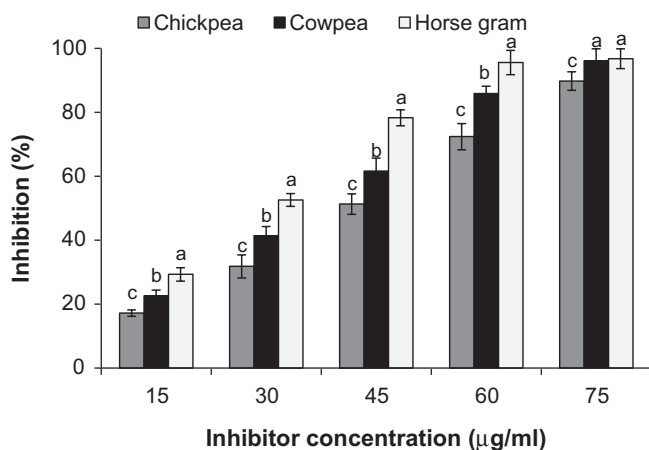


Fig. 1. Trypsin inhibitory activities of chickpea, cowpea and horse gram flour buffer extracts. Trypsin was pre incubated with different concentrations of extract at 37 °C for 10 min and assayed for remaining activity. The data points are the means \pm SD from three experiments. Values marked with different letters (a, b, c) are significantly different ($p < 0.05$).

However, in certain situations the effects of inhibitors on protein digestion might be advantageous, e.g. by improving the intact absorption of some therapeutic proteins, such as orally delivered insulin. Moreover, the control of proteases activity is considered to play a decisive role in a wide range of biological processes and malfunctioning related to cancer progression. Several *in vitro* and *in vivo* studies have provided evidence that certain protease inhibitors of legume seeds (Bowman–Birk types of protease inhibitors) are effective at preventing or suppressing carcinogen-induced transformation (Kennedy, 1998). However, the positive or negative effect of all enzyme inhibitors depends on their content in different legumes and on the dose and frequency of consumption. Even though the chickpea, cowpea and horse gram flours contain high levels of protease inhibitors, these could be inactivated by hydrothermal treatment. Such processed legume flours could constitute valuable additions to monogastric diets when supplemented with cereal protein, and specifically wheat flour.

3.3. Functional properties

3.3.1. Protein/nitrogen solubility

To provide useful information on effective utilisation of cowpea and horse gram flours in various food products, the protein solubility of the flours was investigated at pH values ranging from 2 to 10. The differences in protein solubility, as affected by pH, are shown in Fig. 2. The protein solubility (of all three legume flours) was lowest at pH 4–6 and highest at pH 2–3 and 7–10. The statistically significant lowest solubility value observed at pH 4 was 6.14% for the chickpea sample whereas, at the same pH, cowpea and horse gram showed solubility values of 14.9% and 46.8%, respectively. In contrast, the protein solubilities of cowpea (8.19%) and horse gram (16.4%) were lowest at pH 5.0 (Fig. 2). This might represent the isoelectric point region at which protein–protein interactions disfavour solubility when compared to the other pH levels studied. On the other hand, protein solubility ranged between 69.6% and 73.6% at pH 7 with no significant differences observed between flours. There was a marked increase in solubility, attaining maximum values of 96.2%, 94.5% and 93.6% at pH 10.0 for chickpea, cowpea and horse gram, respectively, with no significant differences observed between flours. These results suggest that, at alkaline pH, there is a greater extraction of the soluble proteins. However, at pH 2.0, the solubility obtained for horse gram flour (84.1%) was significantly higher than the values obtained for cowpea (72.3%) and chickpea (67.0%) flours (Fig. 2). Similar findings were reported for raw cowpea and chickpea flours by Ghavidel and Prakash (2006). For food applications, protein solubility is an important parameter that influences the extent of utilisation in

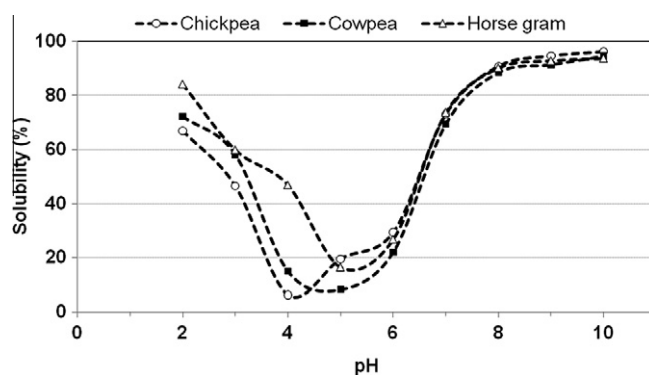


Fig. 2. Effect of pH on nitrogen solubility of flours prepared from chickpea, cowpea and horse gram. The data points are the average values from three independent experiments.

different food matrices. In some cases, such as beverages, high protein solubility is a determinant for application as a fortification ingredient. Therefore, horse gram flour, which exhibited significantly higher solubility at pH 4.0 (46.8%), could make it a very promising candidate for use in acidic beverages. Nevertheless, among the functional properties of proteins, solubility is probably the most critical, because it affects other properties, such as emulsification ability, foam-forming capacity and gel formation. Overall, the protein solubilities of cowpea and horse gram were comparable with chickpea; this could be of interest as chickpea is very widely used as an ingredient in food products.

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

Water absorption capacity of flours plays an important role in the food preparation process because it influences other functional and sensory properties. Furthermore, the range of application of flours as food ingredients is dependent, to a large extent, on their interaction with water. The water absorption capacities of legume flours, under ambient conditions, were in the range 124.6–148.1 g/100 g, where cowpea had the lowest and horse gram the highest WAC (Table 3). The results also showed that the WAC of the horse gram flour was higher than that of chickpea flour. These values are comparable to reported values for dehulled legume flours of green gram (122.6 g/100 g), cowpea (128.5 g/100 g), and chickpea (136.2 g/100 g) (Ghavidel & Prakash, 2006). However, the WAC value 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). It is known that polar amino acid residues of proteins have an affinity for water molecules and differences in WAC of different legumes could be due to the content of these amino acids in legumes. In addition, carbohydrate composition may also be a factor influencing the water-holding capacity of the flours. Flours with high WHC could be good ingredients in bakery applications, such as bread formulations, since a higher WAC enables bakers to add more water to the dough, thus improving the handling characteristics and maintaining freshness in bread. Furthermore, WAC is a critical property of proteins in viscous foods, e.g. soups, dough, custards and baked products, because these are supposed to imbibe water without dissolution of protein, thereby providing body, thickening and viscosity. Similar to the observations made for the WAC values, the highest water solubility index (WSI) value of 8.2% was observed in horse gram flour. The WSI values of chickpea and cowpea flours were found to be 7.4% and 6.8%, respectively (Table 3).

3.3.3. Oil absorption capacity (OAC)

The oil-adsorption capacity of any food compound is important for food applications because it relies mainly on its capacity to physically entrap oil by a complex capillary attraction process. In

Table 3
Functional properties of flours obtained from chickpea, cowpea and horse gram.*

Functional property	Chickpea	Cowpea	Horse gram
Water absorption capacity (g/100 g)	131.6 ± 2.9 ^b	124.6 ± 1.6 ^c	148.1 ± 3.4 ^a
Water solubility index (%)	7.4 ± 0.4 ^b	6.8 ± 0.4 ^b	8.2 ± 0.6 ^a
Oil absorption capacity (g/100 g)	109.3 ± 1.6 ^a	88.3 ± 1.3 ^b	82.4 ± 1.1 ^c
Foaming capacity (%)	46.3 ± 2.1 ^a	43.7 ± 2.4 ^b	41.6 ± 1.8 ^c
Foam stability (%) [#]	39.2 ± 1.7 ^b	43.6 ± 2.3 ^a	37.4 ± 1.7 ^b
Emulsion activity (%)	48.8 ± 0.8 ^b	53.2 ± 1.1 ^b	58.1 ± 0.5 ^a
Emulsion stability (%)	45.1 ± 2.0 ^b	41.0 ± 1.9 ^c	52.0 ± 1.6 ^a

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

* Results are means ± standard deviation of triplicate determinations.

[#] After 30 min.

many food applications, such as emulsion-type meat products, the ability of a food component to entrap oil is an important characteristic because fat acts as a flavour retainer, a consistency trait and an enhancer of mouth feel (Khattab & Arntfield, 2009). The oil absorption capacity of cowpea and horse gram flours was studied and compared with that of chickpea. Significant differences in the oil absorption capacities were noted among the legume flours studied. Chickpea flour had the highest value (109.3 g/100 g), which was followed by cowpea and horse gram flours (Table 3). The oil absorption capacity of legume flours is negatively correlated with water absorption capacity, which is similar to canola meal oil absorption and water absorption capacities reported by Naczek, Diosady, and Rubin (1985). Although cowpea and horse gram flours showed significantly lower OAC values than did chickpea flour, these values are higher than that of the reported values for red bean flour (73.83 g/100 g) (Njintang, Mbofung, & Waldron, 2001) and pigeon pea flour (80.7 g/100 g) (Oshodi & Ekperigin, 1989).

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

Foam formation and stability generally depend on the interfacial film formed by proteins which keeps air bubbles in suspension and slows down the rate of coalescence. Foaming properties are dependent on the proteins, as well as on other components, such as carbohydrates present in the flour. The foaming properties of the chickpea, cowpea and horse gram flours are presented in Table 3. The foams produced by these legume flours were relatively thick with low foam volume but high foam stability after 30 min. The FC and FS values of flours differed significantly. The FC of chickpea was found to be greater than that of cowpea and horse gram (Table 3). Better foaming capacity of chickpea flour implies greater incorporation of air bubbles into the product. Although, a lower value of FC (20.8%) for cowpea flour was reported previously (Akubor, Adamolekun, Oba, Obari, & Abudu, 2003), the observed FC was lower than the 60.2% reported for cowpea protein isolate (Horax, Hettiarachy, Chen, & Jalaluddin, 2004). This may be due to differences in the proteins and the concentrations employed. However, the FC of horse gram is similar to the reported values in the literature (Sreerama, Sashikala, & Pratape, 2008). Cowpea flour showed markedly higher foam stability after 30 min than did chickpea and horse gram flours (Table 3). These results indicate that the proteins and other components of cowpea flour have greater ability to form a strong and cohesive film around air bubbles and greater resistance of air diffusion from the bubbles. In general, all three legume flours depicted high foam stability and may find application in baked and confectionery products.

3.3.5. Emulsifying properties [emulsion activity (Ea) and emulsion stability (Es)]

The emulsion activity reflects the ability and capacity of a protein to aid in the formation of an emulsion and is related to the protein's ability to absorb to the interfacial area of oil and water in an emulsion. The emulsion stability normally reflects the ability of the proteins to impart strength to an emulsion for resistance to stress and changes and is therefore related to the consistency of the interfacial area over a defined time period (Pearce & Kinsella, 1978). The emulsifying activities and emulsion stabilities of chickpea, cowpea and horse gram flours are shown in Table 3. The flour of horse gram was superior to the other flours in emulsifying activity and emulsifying stability (significantly higher than chickpea and cowpea flours). The increased Ea of horse gram flour might be due to the dissociation and partial unfolding of globular proteins, leading to exposure of hydrophobic amino acid residues, which consequently increased the surface activity and adsorption at the oil and water interface. No significant differences were observed between the emulsifying activities of chickpea and cowpea flours. However,

emulsion stability values of all the three flours differed significantly. These results are in concordance with those reported earlier by Mwasaru, Muhammad, Bakar, Yaakob, and Man (2000); who calculated 48.16% and 54.90% emulsion activity and stability, respectively for cowpea protein isolates. However, Ragab, Babiker, and Eltinay (2004) reported the emulsion activity value of 50% and stability value of 82% for cowpea protein isolates. Perhaps differences in the chemical compositions of cowpea cultivars and protein solubility might have accounted for the observed differences in Ea and Es.

4. Conclusions

The results of this study indicate that cowpea and horse gram flours are rich in protein, carbohydrates, resistant starch and dietary fibres. Although chickpea flour has a higher fat content, the overall nutrient composition of these little-known legume flours is comparable to the extensively used chickpea flour, suggesting that these legume flours could serve as cheap and alternate source of proteins. Nitrogen solubility of flours was good at both acid and alkaline pHs. Although oil-absorption and foaming capacities were higher in chickpea flour, better foam stability was observed in cowpea flour. The horse gram flour had the highest water absorption capacity, emulsion activity and emulsion stability. These favourable nutritional and functional properties of cowpea and horse gram flours could be exploited in the preparation and development of food products, such as bakery products, soups, extruded products and ready-to-eat snacks. The flours from these underutilised legumes may also be very attractive for producing composite flours as partial substitutes of chickpea flour in snacks, confectionery and other traditional food products. However, only basic information on nutrition and flour functionality, based on simple model systems, is provided in this study. Further studies are in progress to understand the inherent complexity of protein functionality of these flours, in composite flours and in food systems.

Acknowledgement

We thank Dr. R. Ravi, Sensory Science Department, Central Food Technological Research Institute for statistical evaluation of the data.

References

- Akubor, P. I., Adamolekun, F. O., Oba, C. A., Obari, H., & Abudu, I. O. (2003). Chemical composition and functional properties of cowpea and plantain flour blends for cookie production. *Plant Foods for Human Nutrition*, 58, 1–9.
- Alajaji, S. A., & El-Adawy, T. A. (2006). Nutritional composition of chickpea (*Cicer arietinum* L.) as affected by microwave cooking and other traditional cooking methods. *Journal of Food Composition and Analysis*, 19, 806–812.
- Anderson, R. A., Conway, H. F., Pfeifer, V. F., & Griffin, E. L. (1969). Roll and extrusion – cooking of grain sorghum grits. *Cereal Science Today*, 14, 372–380.
- Asp, N. G., Claves, G., Johnson, C. J., Halmer, H., & Siljestrom, M. (1983). Rapid enzymatic assay of insoluble and soluble dietary fibre. *Journal of Agricultural Food Chemistry*, 31, 476–482.
- Association of Official Analytical Chemists. (1990). *Official Methods of Analysis*, 15th ed.; AOAC: Washington, DC.
- Boye, J. I., Aksay, S., Roufik, S., Ribéreau, S., Mondor, M., Farnworth, E., et al. (2010). Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques. *Food Research International*, 43, 537–546.
- Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Analytical Biochemistry*, 72, 248–254.
- Chau, C. F., & Cheung, P. C. K. (1998). Functional properties of flours prepared from three Chinese indigenous legume seeds. *Food Chemistry*, 61, 429–433.
- Costa, G. E. A., Queiroz-Monici, K. S., Reis, S. M. P. M., & Oliveira, A. C. (2006). Chemical composition, dietary fiber and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. *Food Chemistry*, 94, 327–330.
- Diwakar, P., Kushwah, A., & Kushwah, H. S. (1996). Effect of processing on the functional properties of some local varieties of horse gram (*Dolichos biflorus* L.) in Madhya Pradesh. *Journal of Food Science and Technology (India)*, 33, 150–152.
- Duncan, D. B. (1955). Multiple range and multiple F-test. *Biometrics*, 11, 1–42.
- Ghavidel, R. A., & Prakash, J. (2006). Effect of germination and dehulling on functional properties of legume flours. *Journal of the Science of Food and Agriculture*, 86, 1189–1195.
- Haug, W., & Lantzsich, H.-J. (1983). Sensitive method for the rapid determination of phytate in cereals and cereal products. *Journal of the Science of Food and Agriculture*, 34, 1423–1426.
- Hopwell, R., Yeater, R., & Ullrich, I. (1993). Soluble fiber: Effect on carbohydrate and lipid metabolism. *Progress in Food and Nutrition Science*, 17, 159–182.
- Horax, R., Hettiarachchy, N. S., Chen, P., & Jalaluddin, A. M. (2004). Functional properties of protein isolate from cowpea (*Vigna unguiculata* L. Walp.). *Journal of Food Science*, 69, 119–121.
- Igbedioh, S. O., Olugbemi, K. T., & Akpapunam, M. A. (1994). Effects of processing methods on phytic acid level and some constituents in bambara groundnut (*Vigna subterranea*) and Pigeon pea (*Cajanus cajan*). *Food Chemistry*, 50, 147–151.
- Jacobs, D. R., Jr., & Steffen, L. M. (2003). Nutrients, foods, and dietary patterns as exposures in research: A framework for food synergy. *American Journal of Clinical Nutrition*, 78, 508S–513S.
- Kadam, S. S., & Salunkhe, D. K. (1985). Nutritional composition, processing, and utilization of horse gram and moth bean. *Critical Reviews in Food Science and Nutrition*, 22, 1–26.
- Kakade, M. L., Rackis, J. J., McGhee, J. E., & Puski, G. (1974). Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. *Cereal Chemistry*, 51, 376–382.
- Kehrer, J. P. (1993). Free radicals as mediators of tissue injury and disease. *Critical Reviews in Toxicology*, 23, 21–48.
- Kennedy, A. R. (1998). The Bowman–Birk inhibitor from soybean as an anticarcinogenic agent. *American Journal of Clinical Nutrition*, 68, 1406–1412.
- Khatab, R. Y., & Arntfield, S. D. (2009). Functional properties of raw and processed canola meal. *LWT – Food Science and Technology*, 42, 1119–1124.
- Mangala, S. L., Malleshi, N. G., Mahadevamma & Tharanathan, R. N. (1999). Resistant starch from differently processed rice and ragi (finger millet). *European Food Research and Technology*, 209, 32–37.
- Mwasaru, M. A., Muhammad, K., Bakar, J., Yaakob, B., & Man, C. (2000). Influence of altered solvent environment on the functionality of pigeon pea (*Cajanus cajan*) and cowpea (*Vigna unguiculata*) protein isolates. *Food Chemistry*, 71, 157–165.
- Nacz, M., Diosady, L. L., & Rubin, L. J. (1985). Functional properties of canola meals produced by a two-phase solvent extraction system. *Journal of Food Science*, 50, 1685–1688.
- Nakakuki, T. (2003). Development of functional oligosaccharides in Japan. *Trends in Glycoscience and Glycotechnology*, 15, 57–64.
- National Academy of Sciences (1979). In *Tropical legumes: Resources for the future*, Report of an *Ad hoc* panel of the advisory committee on technology innovation board on science and technology for international development, Washington, D.C.
- Njintang, N. Y., Mbofung, C. M. F., & Waldron, K. W. (2001). In vitro protein digestibility and physicochemical properties of dry red bean (*Phaseolus vulgaris*) flour: Effect of processing and incorporation of soybean and cow pea flour. *Journal of Agricultural and Food Chemistry*, 49, 2465–2471.
- Olaofe, O., Adeyemi, F. O., & Adediran, G. O. (1994). Amino acid, chemical composition and functional properties of some oil seeds. *Journal of Agricultural and Food Chemistry*, 42, 879–881.
- Oshodi, A. A., & Ekperigin, M. M. (1989). Functional properties of pigeon pea (*Cajanus cajan*) flour. *Food Chemistry*, 34, 187–191.
- Pearce, K. N., & Kinsella, J. E. (1978). Emulsifying properties of proteins: Evaluation of a turbidimetric technique. *Journal of Agricultural and Food Chemistry*, 26, 716–723.
- Preet, K., & Punia, D. (2000). Proximate composition, phytic acid, polyphenols and digestibility (*in vitro*) of four brown cowpea varieties. *International Journal of Food Science and Nutrition*, 51, 189–193.
- Prinyawiwatkul, W., Eitenmiller, R. R., Beuchat, L. R., McWatters, K. H., & Phillips, R. D. (1996a). Cowpea flour vitamins and trypsin Inhibitor affected by treatment and fermentation with *Rhizopus microspores*. *Journal of Food Science*, 61, 1039–1042.
- Prinyawiwatkul, W., McWatters, K. H., Beuchat, L. R., & Phillips, R. D. (1996b). Cowpea flour: A potential ingredient in food products. *Critical Reviews in Food Science and Nutrition*, 36, 413–436.
- Rackis, J. J. (1974). Biological and physiological factors in soybeans. *Journal of American Oil Chemists Society*, 51, 161A–174A.
- Ragab, D. M., Babiker, E. E., & Eltinay, A. H. (2004). Fractionation, solubility and functional properties of cowpea (*Vigna unguiculata*) proteins as affected by pH and or salt concentration. *Food Chemistry*, 84, 207–212.
- Reddy, N. R., Pierson, M. D., Sathe, S. K., & Salunkhe, D. K. (1985). Dry bean tannins: A review of nutritional implications. *Journal of American Oil Chemists Society*, 62, 541–553.
- Rehman, Z.-U., & Salariya, A. M. (2005). Effects of hydrothermal processing on antinutrients, protein and starch digestibility of food legumes. *International Journal of Food Science and Technology*, 40, 695–700.
- Shahidi, F., Chavan, U. D., Nacz, M., & Amarowicz, R. (2001). Nutrient distribution and phenolic antioxidants in air-classified fractions of beach pea (*Lathyrus maritimus* L.). *Journal of Agricultural Food Chemistry*, 49, 926–933.
- Siddhuraju, P., & Becker, K. (2007). The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) seed extracts. *Food Chemistry*, 101, 10–19.
- Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods in Enzymology*, 299, 152–178.

- Sosulski, F. W. (1962). The centrifuge method for determining flour absorption in hard red spring wheat. *Cereal Chemistry*, 39, 344–350.
- Sreerama, Y. N., Neelam, D. A., Sashikala, V. B., & Pratapa, V. M. (2010). Distribution of nutrients and antinutrients in milled fractions of chickpea and horse gram: Seed coat phenolics and their distinct modes of enzyme inhibition. *Journal of Agricultural Food Chemistry*, 58, 4322–4330.
- Sreerama, Y. N., Sasikala, V. B., & Pratapa, V. M. (2008). Nutritional implications and flour functionality of popped/expanded horse gram. *Food Chemistry*, 108, 891–899.
- Stanley, D. W., & Aguilera, J. M. (1985). A review of textural defects in cooked reconstituted legumes: The influence of structure and composition. *Journal of Food Biochemistry*, 9, 277–323.
- Turner, B. L., Paphazy, J. M., Haygarth, M. P., & Mckelvie, D. I. (2002). Inositol phosphate in the environment. *Online Journal of Royal Society*, 357, 469–449.
- Yatsumatsu, K., Sawada, K., & Moritaka, S. (1972). Whipping and emulsifying properties of soybean products. *Agricultural Biological Chemistry*, 36, 719–727.