

Characterization of protein isolates from different Indian chickpea (*Cicer arietinum* L.) cultivars

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Abstract

Protein isolates prepared by alkaline solubilization followed by isoelectric precipitation and freeze drying from desi (PBG-1, PDG-4, PDG-3, GL-769, and GPF-2) and kabuli (L-550) chickpea cultivars were evaluated for functional (water and oil absorption capacities, least gelation concentration, foaming capacity and stability) and thermal properties. Significant difference ($P \leq 0.05$) in properties of kabuli and desi chickpea protein isolates was observed. Kabuli chickpea protein isolate showed significantly ($P \leq 0.05$) higher ash (1.14%), protein (94.4%), L^* , ΔE value, oil absorption capacity (OAC) and lower water absorption capacity (WAC) than their corresponding desi chickpea protein isolates. The solubility-pH profile of different protein isolates showed minimum solubility in the pH between 4.0 and 5.0 and two regions of maximum solubility at pH 2.5 and 7.0. Foaming capacity of all protein isolates increased with the increase in concentration. Kabuli chickpea protein isolate showed the highest foam stability (94.7%) after 120 min of storage. The thermal properties of protein isolates from different chickpea cultivars were studied by differential scanning calorimetry (DSC). Protein isolates from both the chickpea types differed significantly ($P \leq 0.05$) in peak denaturation temperature (T_d) and heat of transition (ΔH). Kabuli type protein isolate exhibited lower T_d and ΔH value as compared to those from desi types. The interrelationships between characteristics of protein isolates showed a significant ($P \leq 0.05$) negative correlation of T_d with protein content and OAC. It was also observed that cultivars with high fat content had high ΔH and lower WAC.

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1. Introduction

Chickpea (*Cicer arietinum* L.) is one of the most important legume crops in the Indian sub-continent. Chickpea is the fifth importance in world wide, India being the country which contributes about 75% of the total world production, followed by Turkey, Pakistan, and Mexico as main exporters of high quality grain (Greda, Moreno-Valencia, Falcon-Villa Ma del Refugio, & Barron-Hoyos, 1997). Chickpeas are a good source of protein and carbohydrate and its protein quality is better than other legumes such as pigeon pea, black gram and green gram (Kaur, Singh,

& Sodhi, 2005). The shape, size, and color of chickpea seed vary according to the cultivars. Based on seed color and geographic distribution, chickpea is grouped into two types: desi (Indian origin) and kabuli (Mediterranean and Middle Eastern origin). The seeds of kabuli cultivars are large with white to cream colored seed coat. The seeds of desi cultivars are small, wrinkled with brown, black or green color (Kaur et al., 2005). Substantial differences in these two groups have been observed by several workers with regard to their seed coat percentage, crude fiber content, trace element composition, polyphenol content (Chavan, Kadam, & Salunkhe, 1986; Jambunathan & Singh, 1981; Singh & Jambunathan, 1981), parching properties (Kaur et al., 2005), and properties of their flours (Kaur & Singh, 2005).

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The use of legumes assumes significance as a cheap and concentrated source of proteins, due to the high cost of proteins of animal origin and their inaccessibility by the poor section of the population (Tharanathan & Mahadevamma, 2003). Production of purified vegetable protein is gaining increasing commercial importance due to the consumer preferences for vegetable sources of food and cosmetic ingredients. Numerous researchers have reported the preparation and functional properties of protein concentrates and isolates from plant, animal and microbial sources (Aluko & Yada, 1993; Burgess & Kelly, 1979; Naczk, Diosady, & Rubin, 1985; Sanchez-Vioque, Climente, Vioque, Bautista, & Millan, 1999; Sathe, Deshpande, & Salunkhe, 1982a; Sathe, Deshpande, & Salunkhe, 1982b; Sathe & Salunkhe, 1981; Venkatesh & Prakash, 1993). Legume seeds have been shown to contain high molecular-weight oligomeric storage proteins which are the major components in protein isolates prepared from the seeds (Derbyshire, Wright, & Boulter, 1976). The alkaline extraction and subsequent precipitation of the proteins at the isoelectric point is the most usual way to prepare protein isolates in the food industry (Sanchez-Vioque et al., 1999). After alkaline solubilization of proteins and removal of insoluble material by centrifugation, proteins are precipitated at their isoelectric point.

In recent years there has been increasing interest in the functional potential of plant proteins. Legumes have been the focal point of this interest since they contain 18–25% protein (Pawar & Ingle, 1988). Functional properties constitute the major criteria for the adoption and acceptability of proteins in food system and are determined to a large extent by a protein's physicochemical and structural properties. Sanchez-Vioque et al. (1999) investigated the yield, functional properties and composition of protein isolates from chickpea seeds in relation to the possible use of the isolates in the food industry. They reported that isolates with high water and fat absorption are suitable for preparation of cheese, bakery and meat products whereas isolates with good emulsion capacity are suitable for products such as frankfurters or creams.

Differential scanning calorimetry (DSC) is useful in the analysis of the characteristics of thermal transitions in proteins (Kitabatake, Tahara, & Doi, 1990). Protein denaturation involves structural or conformational changes from native structure without alteration of the amino acid sequence (Zhong & Sun, 2000). The native-to-denatured change in the protein state is a cooperative phenomenon that is accompanied by significant heat uptake, seen as an endothermic peak in the DSC thermogram (Biliaderis, 1983). The onset temperature (T_m) and peak denaturation temperature (T_d) are a measure of protein denaturation (Biliaderis, 1983) and are influenced by the heating rate and the protein concentration (Escamilla-Silva, Guzman-Maldonado, Cano-Medinal, & Gonzalez-Alatorre, 2003). Thermal characteristics of many plant proteins such as oat globulin (Harwalker & Ma, 1987), soybean proteins

(Hermansson, 1979), red bean globulin (Meng & Ma, 2000), and fababean proteins (Arntfield & Murray, 1981) have been studied using DSC. Paredes-Lopez, Ordorica-Falomir, and Olivares-Vazquez (1991) studied the effect of isolation procedure on chickpea protein thermal properties using DSC. Their studies revealed that the preparation procedure affected the thermal behavior of chickpea isolates.

Earlier investigations from our laboratories (Kaur & Singh, 2005), indicated significant difference ($P \leq 0.05$) between physicochemical, functional, thermal and pasting properties of desi and kabuli chickpea flours. The present investigation was undertaken to compare certain physicochemical, functional and thermal properties of protein isolates derived from desi and kabuli Indian chickpea cultivars.

2. Materials and methods

2.1. Materials

Representative samples of six improved commercial chickpea cultivars viz. PBG-1, PDG-4, PDG-3, GL-769, GPF-2 and L-550 from 2002 harvest were obtained from Punjab Agricultural University, Ludhiana, India. The cultivars PBG-1, PDG-4, PDG-3, GL-769, GPF-2 are of desi type while L-550 cultivar is of kabuli type. Seeds of different chickpea cultivars were ground to pass through the sieve no. 72 (British Sieve Standards) to obtain flour. The flour samples were defatted by solvent extraction process using *n*-hexane and then dried at temperature of 50 °C in a hot air cabinet drier and after cooling were packed in air tight containers.

2.2. Preparation of protein isolates

Protein isolates from different chickpea cultivars were prepared using the method described by Johnson and Brekke (1983), as modified by El-Adawy (1996). Dispersions of defatted chickpea flours (5%, w/v) in distilled water were adjusted to pH 9 with 0.1 N NaOH at room temperature (~30 °C), shaken for 1 h and centrifuged at 8000g for 15 min. In order to obtain increased yields, the extraction and centrifugation procedures were repeated twice on the residue. The extracts were combined and the pH adjusted to 4.5 with 1 N HCl to precipitate the protein. The proteins were recovered by centrifugation at 8000g for 15 min, followed by removal of the supernatant by decantation. Protein curd was washed twice with distilled water and centrifuged at 8000g for 10 min. The washed precipitate was then freeze dried as protein isolate.

2.3. Proximate composition

Samples were estimated for their moisture, ash, fat and protein ($N \times 6.25$) content by employing the standard methods of analysis (AOAC, 1990).

2.4. Protein solubility

Protein solubility of samples was studied in the pH range of 2.5–7.0. Sample (100 mg) for each pH was suspended in 20 ml distilled water and the pH of the suspensions was adjusted to a specific value using 0.1 N HCl or NaOH solutions. These suspensions were agitated over a metabolic shaker for 1 h at room temperature; the pH was checked and adjusted, then centrifuged at 8000g for 15 min. The protein content of supernatant was determined by the method of Lowry, Rosebrough, Farr, and Randall (1951) using Bovine Serum Albumin as standard. Triplicate determinations were carried out and solubility profile was obtained by plotting averages of protein solubility (%) against pH. Solubility was expressed as the percentage of the total protein of the original sample that was present in the soluble fraction.

2.5. Color characteristics

Color measurements of samples were carried out using a Hunter colorimeter Model D 25 optical Sensor (Hunter Associates Laboratory Inc., Reston, VA., USA) on the basis of L^* , a^* and b^* values. A glass cell containing sample was placed above the light source, covered with a white plate and L^* , a^* and b^* color values were recorded. The instrument ($45^\circ/0^\circ$ geometry, 10° observer) was calibrated against a standard red colored reference tile ($L_s = 25.54$, $a_s = 28.89$, $b_s = 12.03$). Total color difference (ΔE) was calculated applying the equation

$$\Delta E = [(L_s - L)^2 + (a_s - a)^2 + (b_s - b)^2]^{1/2}$$

The L^* value indicates the lightness, 0–100 representing dark to light. The a^* value gives the degree of the red-green color, with a higher positive a^* value indicating more red. The b^* value indicates the degree of the yellow-blue color, with a higher positive b^* value indicating more yellow.

2.6. Functional properties

2.6.1. Water and oil absorption

Water absorption was measured by the centrifugation method of Sosulski (1962). The sample (3.0 g) was dispersed in 25 ml of distilled water and placed in preweighed centrifuge tubes. The dispersions were stirred after interval of 5 min, held for 30 min, followed by centrifugation for 25 min at 3000g. The supernatant was decanted, excess moisture was removed by draining for 25 min at 50°C , and sample was reweighed. For the determination of fat absorption the method of Lin, Humbert, and Sosulski (1974) was used. Samples (0.5 g) were mixed with 6 ml of corn oil in preweighed centrifuge tubes. The contents were stirred for 1 min with a thin brass wire to disperse the sample in the oil. After a holding period of 30 min, the tubes were centrifuged for 25 min at 3000g. The separated oil was then removed with a pipette and the tubes were

inverted for 25 min to drain the oil prior to reweighing. The water and oil absorption capacities were expressed as grams of water or oil bound per gram of the sample on a dry basis.

2.6.2. Least gelation concentration (LGC)

The LGC was determined by the method of Sathe et al. (1982b). Test tubes containing suspensions of 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18%, and 20% (w/v) material in 5 ml distilled water were heated for 1 h in boiling water followed by rapid cooling under cold running water. The tubes were further cooled at 4°C for 2 h. LGC is the concentration above which the sample did not fall down or slip when the test tube was inverted.

2.6.3. Foaming capacity (FC) and foam stability (FS)

The capacity and stability of foams were determined by the method of Lin et al. (1974). 50 ml of 3% (w/v) dispersions of sample in distilled water were homogenized using homogenizer (Yorco, India) at high setting for 2–3 min. The blend was immediately transferred into a graduated cylinder and the homogenizer cup was rinsed with 10 ml distilled water, which was then added to the graduated cylinder. The volume was recorded before and after whipping. FC was expressed as the volume (%) increase due to whipping. For the determination of FS, foam volume changes in the graduated cylinder were recorded at intervals of 20, 40, 60, and 120 min of storage. To study the effect of concentration on foamability; 2%, 4%, 5%, 7% and 10% (w/v) aqueous suspensions of chickpea protein isolates were whipped identically as described above and the final volume was noted in each case in a graduated cylinder.

2.7. Thermal properties

Thermal characteristics of chickpea protein isolates were analyzed using a Differential Scanning Calorimeter (DSC-821 $^\circ$, Mettler Toledo, Switzerland) equipped with a thermal analysis data station. Freeze dried protein isolates (2.0 mg each) were accurately weighed in an aluminium pan (Mettler, ME-27331) and 10 μl of phosphate buffer was added. Samples were hermetically sealed and allowed to stand for 1 h at room temperature before heating in DSC. The DSC analyzer was calibrated using indium and an empty sealed aluminium pan was used as reference. Sample pans were heated at a programmed rate of $10^\circ\text{C}/\text{min}$ from 30 to 130°C . Onset temperature (T_m), peak denaturation temperature (T_d), and heat of transition or enthalpy of denaturation (ΔH) were calculated automatically.

2.8. Statistical analysis

The data reported in all the tables are an average of triplicate observations and were subjected to one-way analysis of variance (ANOVA) using Minitab Statistical Software version 13 (Minitab Inc., USA).

3. Results and discussion

3.1. Proximate composition

Proximate composition of the seed flours and the protein isolates from different chickpea cultivars are presented in Table 1. The ash content of flours and protein isolates from different chickpea cultivars ranged between 2.72–2.91% and 0.82–1.14%, respectively. Ash content of 2.9% in chickpea protein isolates (Sanchez-Vioque et al., 1999) and 0.71% in lupin seed protein concentrates (Sathe et al., 1982b) has been reported. Although the chickpea flours were extracted with hexane, lipids were not removed completely and the part of the same remained in the flours (0.53–1.21%). These lipids mainly of a polar nature interacted with proteins (Kikugawa, Ido, & Mikami, 1981). The protein isolates prepared from defatted flours showed fat content of 0.49–0.98%. Protein content of isolates obtained from different chickpea cultivars ranged between 89.9% and 94.4%, the lowest for GL-769 and the highest for L-550 (kabuli) chickpea was observed. Both flour and protein isolate from L-550 had significantly ($P \leq 0.05$) higher ash and protein content than those from desi types. The fat and protein content of chickpea isolates in the present study were comparable to those reported for cowpea and pigeon pea protein isolates (Mwasaru, Muhammad, Bakar, & Cheman, 1999).

3.2. Color characteristics

Hunter Lab color values (L^* , a^* , b^* and ΔE) of protein isolates from different chickpea cultivars are shown in Table 2. The varietal difference was observed for various Hunter color parameters. The Hunter values showed that chickpea isolates were significantly ($P \leq 0.05$) darker and reddish in color, with lower L^* value (58.63–61.33), and higher a^* value as compared to chickpea flours (Kaur & Singh, 2005). Chickpea protein isolates showed a^* and b^* values between 1.88–2.21 and 22.46–24.95, respectively.

Table 1
Chemical composition (dwb) of defatted flours and protein isolates from different chickpea cultivars^{a,b}

Cultivar	Defatted chickpea flours			Chickpea protein isolates		
	Ash (%)	Fat (%)	Protein (%) ^c	Ash (%)	Fat (%)	Protein (%) ^c
<i>Desi type chickpea</i>						
PBG-1	2.72a	0.96b	23.7b	0.89a	0.83b	94.3b
PDG-4	2.77a	0.53a	20.6a	0.96b	0.49a	92.8ab
PDG-3	2.83ab	1.17bc	23.9b	1.04bc	0.98c	93.3ab
GL-769	2.84ab	1.16bc	24.3bc	0.82a	0.85b	89.9a
GPF-2	2.88b	1.17bc	22.3ab	0.99b	0.92bc	91.6ab
<i>Kabuli type chickpea</i>						
L-550	2.91b	1.21c	26.7c	1.14c	0.94bc	94.4b

^a Means followed by same letter within a column do not differ significantly ($P > 0.05$).

^b Mean of triplicate analyses.

^c Total nitrogen $\times 6.25$.

Table 2
Hunter color values of protein isolates from different chickpea cultivars^{a,b}

Cultivar	L^*	a^*	b^*	ΔE^c
<i>Desi type chickpea</i>				
PBG-1	58.91ab	1.99b	24.95c	44.77a
PDG-4	58.63a	2.21c	23.79b	44.10a
PDG-3	59.32ab	1.93ab	22.46a	44.46a
GL-769	59.08ab	2.04c	24.05bc	44.61a
GPF-2	59.92b	1.96b	23.62ab	45.18ab
<i>Kabuli type chickpea</i>				
L-550	61.33c	1.88a	24.91c	46.65b

^a Means followed by same letter within a column do not differ significantly ($P > 0.05$).

^b Mean of triplicate analyses.

^c Total color difference.

ΔE , which indicated total color difference, for different chickpea protein isolates ranged from 44.10 to 46.65. Protein isolate from kabuli chickpea showed the highest L^* (61.33) and ΔE (46.65) value, indicating its lighter color as compared to isolates from desi types. (Paredes-Lopez et al., 1991) reported L^* , a^* , b^* and ΔE value of 56.8, 3.5, 17.2 and 39.5, respectively for chickpea protein isolates, measured using a white standard, in place of red standard used in the present study. Pigmentation in legume protein isolates can be controlled by carrying out an aqueous pre-extraction at pH 5.5 (Paredes-Lopez et al., 1991), addition of sodium metabisulfite to the extracting medium (Mansour, Peredi, & Dworschak, 1992) and by dehulling the raw material prior to the extraction (Onigbinde & Onobun, 1993).

3.3. Protein solubility

Protein solubility at different pH may serve as a useful indicator of the performance of protein isolates in the food systems, and also the extent of protein denaturation because of heat or chemical treatment (Horax, Hettiarachchy, Chen, & Jalaluddin, 2004a). The solubility profiles of desi and kabuli chickpea protein isolates did not differ significantly ($P \leq 0.05$), in which the isolates showed minimum solubility in the pH range 4.0–5.0, essentially the isoelectric pH range and two regions of maximum solubility at pH 2.5 and 7.0 (Fig. 1). Vani and Zayas (1995) reported that most of the plant proteins have isoelectric pH at 4.0–5.0. At the isoelectric point, there is no net charge on the protein; as a result there are no repulsive interactions and the protein–protein interactions disfavor solubility (Singh, Kaur, & Sandhu, 2005). At low pH, large net charges are induced and repulsive forces increase, resulting in unfolding of proteins. Above pH 6.5, all proteins had solubility greater than 70%. These observations are in agreement with those reported earlier for chickpea (Sanchez-Vioque et al., 1999), lentil (Bora, 2002), soy protein (Achouri, Zhang, & Shying, 1998) and rapeseed (Goncalves et al., 1997). Profiles with low solubility over a broad range of pH are indicative of severe protein denaturation and insolubilization (Hermansson,

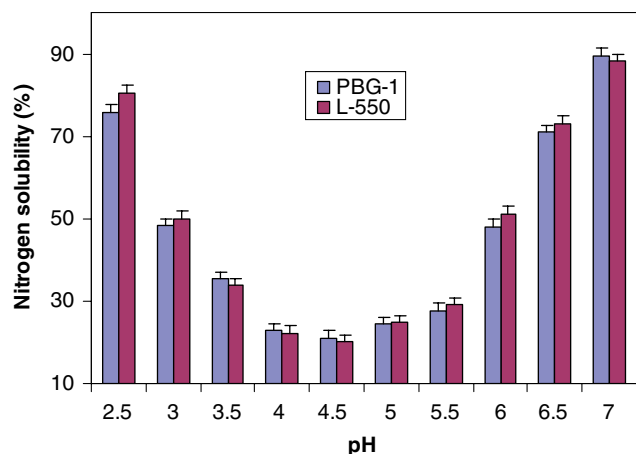


Fig. 1. Protein solubility profiles of isolates from different chickpea cultivars.

1979; Kinsella, 1979; Lillford, 1983; Nakai, 1983) which have been shown to markedly affect the functional properties of proteins.

3.4. Functional properties

The functional properties of protein isolates primarily determine their utility in food products. The genotypes belonging to two distinct chickpea groups showed large difference in certain functional properties. The protein isolates from different chickpea cultivars exhibited higher water absorption capacity (WAC) as compared to their flours (Fig. 2). This might be due to the fact that the isolates have great ability to swell, dissociate and unfold, exposing additional binding sites, whereas the carbohydrate and other components present in flours may impair it (Kinsella, 1979). Protein isolate from kabuli chickpea showed significantly ($P \leq 0.05$) lower WAC (2.34 g/g) than those obtained from desi chickpea cultivars. The lower WAC of kabuli chickpea protein isolate could be due to the low availability of polar amino acids as the latter have been shown to be primary sites for water interaction of

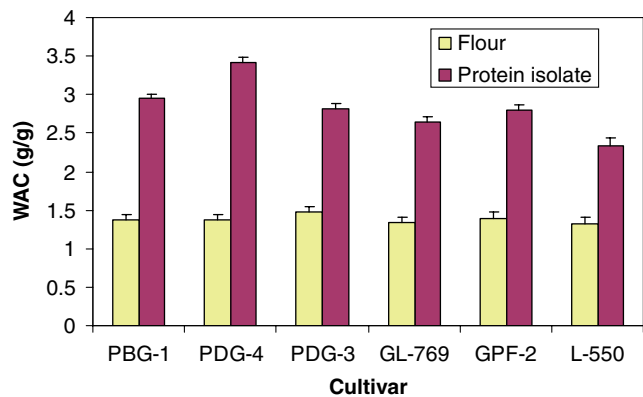


Fig. 2. Water absorption capacity (WAC) of flour and protein isolates from different chickpea cultivars.

proteins (Kuntz, 1971). WAC of protein isolates in the present study compared favorably to isolates from great northern bean (2.73 g/g) (Sathe & Salunkhe, 1981), safflower (1.80–2.82 ml/g) (Paredes-Lopez & Ordorica-Falomin, 1986), rapeseed (1.33 g/g) (Mansour et al., 1992), winged bean protein concentrate (3.52 g/g) (Sathe et al., 1982a), faba bean (1.84 ml/g) and chickpea (1.88 ml/g) (Abdel-Aal, Shehata, Mahdy, & Youssef, 1986). Difference in the content of non-proteinaceous materials of the isoelectric isolates may also have contributed to the observed difference in water absorption as previously observed for isolates from adzuki bean (Tjahjadi, Lin, & Breene, 1988), sunflower (Kilara, Humbert, & Sosulski, 1972) and great northern bean (Sathe & Salunkhe, 1981). It was also observed that isolates with high fat content had lower WAC ($r = -0.823$, $P \leq 0.05$).

The oil absorption capacity (OAC) is of great importance from an industrial viewpoint, since it reflects the emulsifying capacity, a highly desirable characteristic in products such as mayonnaise (Escamilla-Silva et al., 2003). OAC of protein isolates ranged between 2.08 and 3.96 g/g (Fig. 3), which were significantly ($P \leq 0.05$) higher than those observed for their corresponding flours (1.05–1.24 g/g). Kabuli chickpea protein isolate exhibited significantly ($P \leq 0.05$) higher OAC than the desi chickpea protein isolates, suggesting the presence of more non polar amino acids in kabuli protein. The presence of several non polar side chains may bind the hydrocarbon chains of fats, thereby resulting in higher absorption of oil (Sathe et al., 1982a). OAC of protein isolates in the present study were comparable to commercial soy isolate (3.29 ml/g), and winged bean protein concentrate (4.01 g/g) as reported earlier (Mwasaru et al., 1999; Sathe et al., 1982a). Lower OAC of 1.7 ml/g for chickpea isolates (Paredes-Lopez et al., 1991), 1.59–2.58 ml/g for adzuki bean isolates (Tjahjadi et al., 1988) and 2.0–2.22 ml/g for cowpea protein isolates (Sefa-Dedeh & Yiadom-Farkye, 1988) has been reported. High OAC of the protein is required in ground meal formulation, meat replacers and extenders, doughnuts, baked goods and soups.

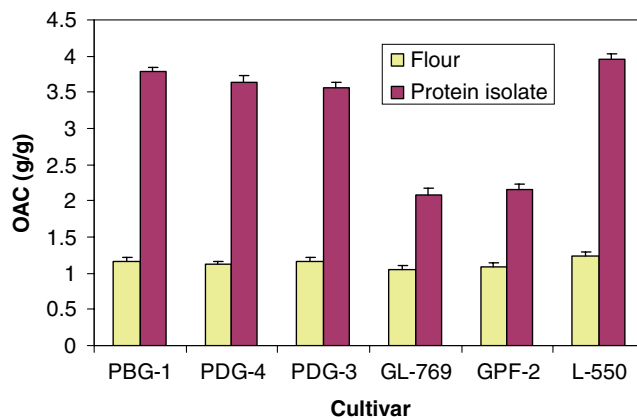


Fig. 3. Oil absorption capacity (OAC) of flour and protein isolates from different chickpea cultivars.

Least gelation concentration (LGC) indicates the gelation capacity, the lower the LGC the better is the gelling ability of proteins (Akintayo, Oshodi, & Esuoso, 1999). LGC for various chickpea protein isolates ranged between 14% and 18% (Table 3), whereas the seed flours presented lower gelation concentration of 10–14%. In this sense, the gelation is not only a function of protein quantity but seems also to be related to the type of protein as well as to non-protein components, as suggested in studies on the Great northern bean and adzuki bean (Sathe & Salunkhe, 1981; Tjahjadi et al., 1988). PDG-4 (desi chickpea) protein isolate formed a firm gel at a significantly ($P \leq 0.05$) lower concentration (14%), suggesting its better gelling ability than other legume protein isolates. LGC of 8% for great northern bean protein concentrate (Sathe & Salunkhe, 1981), 12% for cow pea (Horax et al., 2004a), 10% for mung bean protein isolate (Coffman & Garcia, 1977), 14% for lupin seed protein (Sathe et al., 1982b), and 12% for mucuna bean protein (Adebowale & Lawal, 2003) has been reported. Circle and Smith (1972) reported that firm and resistant gels are formed from soy protein isolates at 16–17% concentrations.

The foaming capacity (FC) and foam stability (FS) are used as indices of the whipping properties of protein isolates (Mwasaru et al., 1999). Proteins foam when whipped because of their surface active properties. FC of protein isolates from different chickpea cultivars was observed to be in the range between 30.4% and 44.3%, which were significantly ($P \leq 0.05$) higher than their corresponding flours (Fig. 4). FC of 36% for winged bean protein concentrate (Sathe et al., 1982a), 235% for soy isolates (Lin et al., 1974), 58% for mucuna bean protein concentrate (Adebowale & Lawal, 2003), 32% for lupin seed (Sathe et al., 1982b), and 80% for pigeon pea (Akintayo et al., 1999) has been reported. Graham and Phillips (1976) linked good foamability with flexible protein molecules that can reduce surface tension, while a globular protein which is relatively difficult to surface denature gives low foamability. The major proteins of legumes are also globular in nature which may be difficult to surface denature, hence resulting in lower foaming properties (Sathe et al., 1982a). Foaming

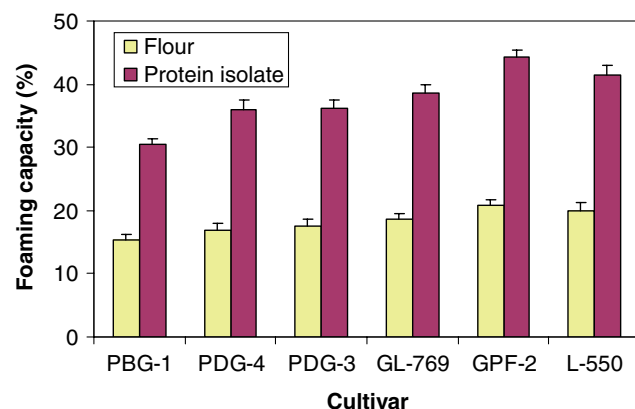


Fig. 4. Foaming capacity (%) of flour and protein isolates from different chickpea cultivars.

capacity was dependent on sample concentration (Fig. 5). All the protein isolates showed progressive increasing foamability with the increase in concentration of solids. The availability of more protein, as the level of protein isolate increases in the aqueous dispersion, enhances foam formation. There was a rapid increase in foam volume up to 7% (w/v) solids concentration with a maximum at 10% (w/v). The results are in agreement with those obtained earlier on the foaming properties of pigeon pea protein concentrate (Akintayo et al., 1999), lupin seed protein concentrates (Sathe et al., 1982b) and chickpea flours (Kaur & Singh, 2005). The decrease in foam volume as a function of time was observed (Fig. 6). A similar trend has been reported for great northern bean proteins (Sathe & Salunkhe, 1981) and mucuna bean protein concentrates (Adebowale & Lawal, 2003). FS is important since the usefulness of whipping agents depends on their ability to maintain the whip as long as possible (Lin et al., 1974). Kabuli chickpea (L-550) protein isolate showed the highest FS (94.7%) after 120 min of storage. The good FS of all chickpea protein isolates (>85%) suggest that the native proteins that are soluble in the continuous phase (water) are very surface-active in chickpea proteins.

Table 3

Least gelation concentration of chickpea protein isolates after heating in boiling water for 1 h followed by cooling for 2 h at 4 °C^a

Concentration (%)	Desi type chickpea					Kabuli chickpea
	PBG-1	PDG-4	PDG-3	GL-769	GPF-2	L-550
2	–	–	–	–	–	–
4	–	–	–	–	–	–
6	–	–	–	–	–	–
8	–	–	–	–	–	–
10	–	–	–	–	–	–
12	–	–	–	–	–	–
14	–	Gel	–	–	–	–
16	Gel	Firm gel	–	–	–	–
18	Firm gel	Very firm gel	Gel	–	Gel	Gel
20	Very firm gel	Very firm gel	Firm gel	Gel	Firm gel	Firm gel

(–) Indicates no gelation.

^a Mean of triplicate determinations.

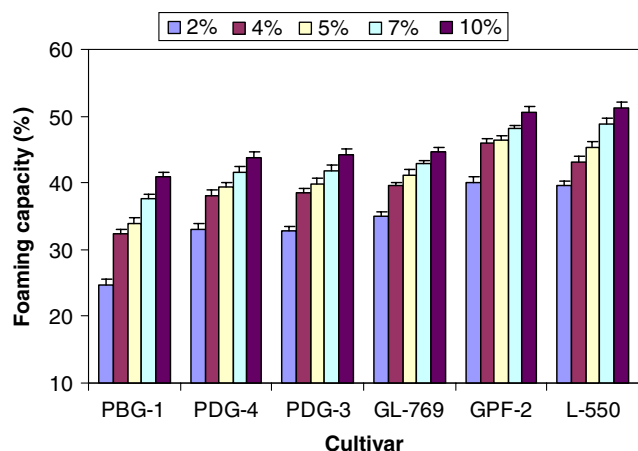


Fig. 5. Effect of protein concentration on foaming capacity (%) of different chickpea cultivars.

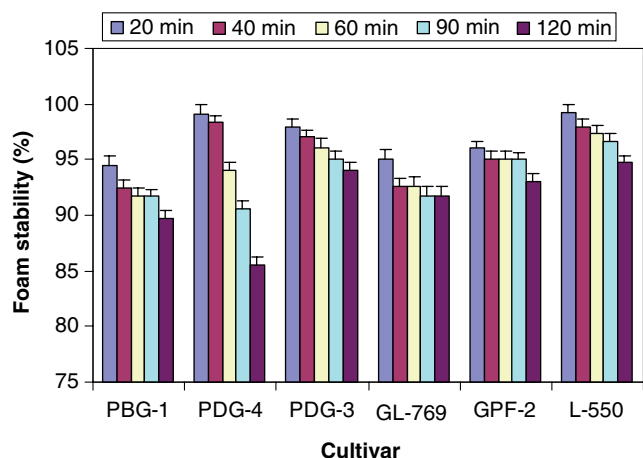


Fig. 6. Foam stability (%) of protein isolates from different chickpea cultivars after 20, 40, 60, 90, and 120 min of storage.

3.5. Thermal properties

Differential scanning calorimetry (DSC) was performed on protein isolates to investigate their thermal stability. The thermal stability of the proteins functionally indicates their resistance to aggregation in response to heating (Horax, Hettiarachchy, Chen, & Jalaluddin, 2004b). The onset temperature (T_m), peak denaturation temperature (T_d) and heat of transition or enthalpy (ΔH) of chickpea protein isolates are shown in Table 4. T_d is the temperature at which a transition occurs and is a measure of thermal stability. T_d for all chickpea protein isolates was less than 100 °C. Seed globulins have been found to possess T_d in the range of 83.8–107.8 °C (Gorinstein, Zemser, & Paredes, 1996; Marcone, Kakuda, & Yada, 1998). Denaturation temperatures of desi chickpea protein isolates ranged between 98.5 and 99.8 °C, and were higher than those from kabuli chickpea protein isolate (98.1 °C). T_d of 114.7 °C for flaxseed protein (Li-Chan & Ma, 2002), 88 °C for chickpea (Paredes-Lopez et al., 1991), 91.4 °C for cowpea and 92 °C

Table 4
Thermal properties of protein isolates from different chickpea cultivars^{a,b}

Cultivar	T_m (°C)	T_d (°C)	ΔH (J/g)
<i>Desi type chickpea</i>			
PBG-1	89.8ab	98.5a	3.88b
PDG-4	90.9bc	99.3ab	5.83c
PDG-3	86.4a	98.6a	5.19bc
GL-769	91.7c	99.8b	4.35bc
GPF-2	89.6ab	99.1ab	5.46bc
<i>Kabuli type chickpea</i>			
L-550	88.3b	98.1a	2.84a

T_m , onset temperature of denaturation.

T_d , denaturation temperature.

ΔH , enthalpy of denaturation.

^a Mean of triplicate analyses.

^b Means followed by same letter within a column do not differ significantly ($P > 0.05$).

for pigeon pea (Mwasaru et al., 1999) proteins has been reported. The heat stability of proteins is controlled by their balance of polar and non-polar residues (Bigelow, 1967), with higher heat stability (higher T_d) for proteins having higher proportions of non-polar residues. A significant ($P \leq 0.05$) negative correlation of T_d with protein content ($r = -0.918$) and OAC ($r = -0.763$) of chickpea protein isolates was observed. T_m was observed to have a significant ($P \leq 0.05$) positive correlation ($r = 0.742$) with T_d . The transition heat (ΔH) is used to monitor the proportion of the protein that does not denature during the process (Arntfield & Murray, 1981; Biliaderis, 1983). Kabuli protein isolate showed lower ΔH (2.84 J/g) than those from desi types (3.88–5.83 J/g). ΔH of 16.8 J/g for flaxseed protein (Li-Chan & Ma, 2002), 3.9 J/g for chickpea (Paredes-Lopez et al., 1991), 8.42–10.33 J/g for cowpea (Horax et al., 2004b) and 11.21 J/g for pigeon pea (Mwasaru et al., 1999) proteins has been reported. It was observed that isolates with higher WAC showed higher ΔH values. Besides difference in protein structure and composition, interactions of proteins with residual salts in the isolates may have some effect on protein thermal stability (Arakawa & Timasheff, 1982; Murray, Arntfield, & Ismound, 1985).

4. Conclusion

Protein isolates from desi and kabuli chickpea cultivars differed significantly in their functional and thermal properties. Chickpea protein isolates showed higher protein content with respect to their flours, hence are suitable to provide additional protein in various high protein products. Kabuli chickpea protein isolate differed significantly from desi types with respect to water and oil absorption, gelation capacity, foaming properties, denaturation temperature and enthalpy of denaturation. Protein solubility profiles showed a decreasing solubility with increasing pH until it reached a minimum at the isoelectric point (pH 4.0–5.0). Significant correlations of T_d with protein, T_m ,

and OAC and of ΔH with WAC of protein isolates were observed.

References

- Abdel-Aal, E. S. M., Shehata, A. A., Mahdy, E. A. R., & Youssef, M. M. (1986). Extractability and functional properties of some legume proteins isolated by three methods. *Journal of the Science of Food and Agriculture*, *37*, 553–559.
- Achouri, A., Zhang, W., & Shying, X. U. (1998). Enzymatic hydrolysis of soy protein isolate and effect of succinylation on functional properties of resulting protein hydrolysates. *Food Research International*, *31*, 617–623.
- Adebowale, K. O., & Lawal, O. S. (2003). Foaming, gelation and electrophoretic characteristics of mucuna bean (*Mucuna pruriens*) protein concentrate. *Food Chemistry*, *83*, 237–246.
- Akintayo, E. T., Oshodi, A. A., & Esuoso, K. O. (1999). Effect of ionic strength and pH on the foaming and gelation of pigeon pea (*Cajanus cajan*) protein concentrates. *Food Chemistry*, *66*, 51–56.
- Aluko, R. E., & Yada, R. Y. (1993). Relationship of hydrophobicity and solubility with some functional properties of cowpea (*Vigna unguiculata*) protein isolate. *Journal of the Science of Food and Agriculture*, *62*, 331–335.
- AOAC (1990). *Official methods of analysis* (15th ed.). Washington, DC: Association of Official Analytical Chemists.
- Arakawa, J., & Timasheff, S. N. (1982). Preferential interactions of proteins with salts in concentrated solutions. *Biochemistry*, *24*, 6545–6552.
- Arntfield, S. D., & Murray, E. D. (1981). The influence of processing parameters on food protein functionality (I) Differential scanning calorimetry as indicator of protein denaturation. *Canadian Institute of Food Science and Technology*, *14*, 289–294.
- Bigelow, C. C. (1967). On the average hydrophobicity of proteins and the relationship between it and protein structure. *Journal of Theoretical Biology*, *16*, 187–211.
- Biliaderis, C. G. (1983). Differential scanning calorimetry in food research. A review. *Food Chemistry*, *10*, 239–265.
- Bora, P. S. (2002). Functional properties of native and succinylated lentil (*Lens culinaris*) globulins. *Food Chemistry*, *77*, 171–176.
- Burgess, K. J., & Kelly, J. (1979). Technical note: selected functional properties of a whey protein isolate. *Journal of Food Technology*, *14*, 325.
- Chavan, J. K., Kadam, S. S., & Salunkhe, D. K. (1986). Biochemistry and technology of chickpea (*Cicer arietinum* L.) seeds. *CRC Critical Reviews in Food Science and Nutrition*, *25*, 107–132.
- Circle, S. J., & Smith, A. K. (1972). Functional properties of commercial edible soybean products. In G. E. Inglett (Ed.). *Symposium on seed proteins*. Westport, CT: Avi Publishing Co.
- Coffman, C. W., & Garcia, V. V. (1977). Functional properties and amino acid content of a protein isolate from mung bean flour. *Journal of Food Technology*, *12*, 473–484.
- Derbyshire, E., Wright, D. J., & Boulter, D. (1976). Legumin and vicillin storage proteins of legume seeds. *Phytochemistry*, *15*, 3–24.
- El-Adawy, T. A. (1996). Chemical, nutritional and functional properties of mung bean protein isolate and concentrate. *Menofiya Journal of Agricultural Research*, *21*, 657–672.
- Escamilla-Silva, E. M., Guzman-Maldonado, S. H., Cano-Medinal, A., & Gonzalez-Alatorre, G. (2003). Simplified process for the production of sesame protein concentrate. Differential scanning calorimetry and nutritional, physicochemical and functional properties. *Journal of the Science of Food and Agriculture*, *83*, 972–979.
- Goncalves, N., Vioque, J., Clemente, A., Sanchez-Vioque, R., Bautista, J., & Millan, F. (1997). Obtencion y caracterizacion de aislados proteicos de colza. *Grasas y aceites*, *48*, 282–289.
- Gorinstein, S., Zemser, M., & Paredes, L. O. (1996). Structural stability of globulins. *Journal of Agricultural and Food Chemistry*, *44*, 100–105.
- Graham, D. E., & Phillips, M. C. (1976). The conformation of proteins at the air–water interface and their role in stabilizing of foam. In R. J. Akers (Ed.). *Foams* (pp. 237). London: Academic Press.
- Greda, A. Y. F., Moreno-Valencia, J. G., Falcon-Villa Ma del Refugio & Barron-Hoyos, J. M. (1997). Isolation and partial characterization of starches from dry beans (*Phaseolus vulgaris*) and chickpeas (*Cicer arietinum*) grown in Sonora, Mexico. *Starch*, *49*, 341–345.
- Harwalker, V. R., & Ma, C.-Y. (1987). Study of thermal properties of oat globulin by differential scanning calorimetry. *Journal of Food Science*, *52*, 394–398.
- Hermansson, A. M. (1979). Methods of studying functional characteristics of vegetable proteins. *Journal of American Oil Chemists Society*, *56*, 272–279.
- Horax, R., Hettiarachchy, N. S., Chen, P., & Jalaluddin, M. (2004a). Functional properties of protein isolate from cowpea (*Vigna unguiculata* L. Walp.). *Journal of Food Science*, *69*, 119–121.
- Horax, R., Hettiarachchy, N. S., Chen, P., & Jalaluddin, M. (2004b). Preparation and characterization of protein isolate from cowpea (*Vigna unguiculata* L. Walp.). *Journal of Food Science*, *69*, 114–118.
- Jambunathan, R., & Singh, U. (1981). Studies on desi and kabuli chickpea (*Cicer arietinum*) cultivars 3. Mineral and trace elements composition. *Journal of Agricultural and Food Chemistry*, *29*, 1091–1093.
- Johnson, E. A., & Brekke, J. (1983). Functional properties of acylated pea protein isolates. *Journal of Food Science*, *48*, 722–725.
- Kaur, M., & Singh, N. (2005). Studies on functional, thermal and pasting properties of flours from different Chickpea (*Cicer arietinum* L.) cultivars. *Food Chemistry*(91/3), 403–411.
- Kaur, M., Singh, N., & Sodhi, N. S. (2005). Physicochemical, cooking, textural and roasting characteristics of Chickpea (*Cicer arietinum* L.) cultivars. *Journal of Food Engineering*, *69*, 511–517.
- Kikugawa, K., Ido, Y., & Mikami, A. (1981). Studies on peroxidized lipids. VI. Fluorescents products derived from the reaction of primary amines, malonaldehyde and monofunctional aldehydes. *Journal of American Oil Chemists Society*, *61*, 1574–1581.
- Kilara, A., Humbert, E. S., & Sosulski, F. W. (1972). Nitrogen extractability and moisture absorption characteristics of sunflower seed products. *Journal of Food Science*, *37*, 771–773.
- Kinsella, J. E. (1979). Functional properties of soy proteins. *Journal of American Oil Chemists Society*, *56*, 242–249.
- Kitabatake, N., Tahara, M., & Doi, E. (1990). Thermal denaturation of soybean protein at low water contents. *Journal of Agricultural Biological Chemistry*, *54*(9), 2205–2212.
- Kuntz, I. D. (1971). Hydration of macro-molecules. III: hydration of polypeptides. *Journal of American Oil Chemists Society*, *93*, 514–516.
- Li-Chan, E. C. Y., & Ma, C. Y. (2002). Thermal analysis of flaxseed (*Linum usitatissimum*) proteins by differential scanning calorimetry. *Food Chemistry*, *77*, 495–502.
- Lillford, P. J. (1983). Extraction processes and their effect on protein functionality. *Plantarum Qualitas Plant Food for Human Nutrition*, *32*, 401–409.
- Lin, M. J. Y., Humbert, E. S., & Sosulski, F. W. (1974). Certain functional properties of sunflower meal products. *Journal of Food Science*, *39*, 368–370.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the folin reagent. *Journal of Biological Chemistry*, *193*, 265.
- Mansour, E. H., Peredi, J., & Dworschak, E. (1992). Preparation and functional properties of rapeseed protein products. *Acta Alimentarius*, *21*, 293–305.
- Marcone, M. F., Kakuda, Y., & Yada, R. Y. (1998). Salt-soluble seed globulins of dicotyledonous and monocotyledonous plants. II. Structural characterization. *Food Chemistry*, *63*, 265–274.
- Meng, G.-T., & Ma, C.-Y. (2000). Thermal properties of *Phaseolus angularis* (red bean) globulin. *Food Chemistry*, *73*, 453–460.
- Murray, E. D., Arntfield, S. D., & Ismound, M. A. H. (1985). The influence of processing parameters on food protein functionality. II. Factors affecting thermal properties and analyzed by differential

- scanning calorimetry. *Canadian Institute of Food Science and Technology Journal*, 18, 158.
- Mwasaru, A. M., Muhammad, K., Bakar, J., & Cheman, Y. B. (1999). Effect of isolation technique and conditions on the extractability, physicochemical and functional properties of pigeon pea (*Cajanus cajan*) and cow pea (*Vigna unguiculata*) protein isolates. II. Functional properties. *Food Chemistry*, 67, 445–452.
- Nacz, M., Diosady, L. L., & Rubin, L. J. (1985). Functional properties of colona meals produced by a two phase solvent extraction system. *Journal of Food Science*, 50, 1685–1692.
- Nakai, S. (1983). Structure–function relationships of food proteins with an emphasis on the importance of protein hydrophobicity. *Journal of Agricultural and Food Chemistry*, 31, 676–683.
- Onigbinde, A. O., & Onobun, V. (1993). Effects of pH on some cooking properties of cowpea (*V. unguiculata*). *Food Chemistry*, 47, 125–127.
- Paredes-Lopez, O., & Ordorica-Falomir, C. (1986). Functional properties of safflower protein isolates: water absorption, whipping and emulsifying characteristics. *Journal of the Science of Food and Agriculture*, 37, 1104–1108.
- Paredes-Lopez, O., Ordorica-Falomir, C., & Olivares-Vazquez, M. R. (1991). Chickpea protein isolates: physicochemical, functional and nutritional characterization. *Journal of Food Science*, 56(3), 726–729.
- Pawar, V. D., & Ingle, U. M. (1988). Functional properties of raw and cooked moth bean (*Phaseolus acontifolius Jacq*) flours. *Journal of Food Science & Technology*, 25(4), 186–189.
- Sanchez-Vioque, R., Climente, A., Vioque, J., Bautista, J., & Millan, F. (1999). Protein isolate from chickpea (*Cicer arietinum* L.): chemical composition, functional properties and protein characterization. *Food Chemistry*, 64, 237–243.
- Sathe, S. K., Deshpande, S. S., & Salunkhe, D. K. (1982a). Functional properties of winged bean (*Psophocarpus tetragonolobus* L. DC) proteins. *Journal of Food Science*, 47, 503–509.
- Sathe, S. K., Deshpande, S. S., & Salunkhe, D. K. (1982b). Functional properties of lupin seeds (*Lupinus mutabilis*) proteins and protein concentrates. *Journal of Food Science*, 47, 491–497, 499–500.
- Sathe, S. K., & Salunkhe, D. K. (1981). Functional properties of great northern bean proteins: emulsion, foaming, viscosity and gelation properties. *Journal of Food Science*, 46, 71–75.
- Sefa-Dedeh, S., & Yiadom-Farkye, N. A. (1988). Some functional characteristics of cowpea (*Vigna unguiculata*), bambara beans (*Voandzeia subterranean*) and their products. *Canadian Institute of Food Science and Technology Journal*, 21, 266–270.
- Singh, U., & Jambunathan, R. (1981). Relationship between nonprotein nitrogen and total nitrogen in chickpea (*Cicer arietinum* L.) seed. *Journal of Agricultural and Food Chemistry*, 29, 423–424.
- Singh, N., Kaur, M., & Sandhu, K. S. (2005). Physicochemical and functional properties of freeze-dried and oven dried corn gluten meals. *Drying Technology*, 23, 1–14.
- Sosulski, F. W. (1962). The centrifuge method for determining flour absorption in hard red spring wheats. *Cereal Chemistry*, 39, 344–350.
- Tharanathan, R. N., & Mahadevamma, S. (2003). Grain legumes – A boon to human nutrition. *Trends in Food Science and Technology*, 14, 507–518.
- Tjahjedi, C., Lin, S., & Breene, W. M. (1988). Isolation and characterization of adzuki bean (*Vigna angularis* cv. *Takara*) proteins. *Journal of Food Science*, 53, 1438–1443.
- Vani, B., & Zayas, J. F. (1995). Wheat germ protein flour solubility and water retention. *Journal of Food Science*, 60, 845–848.
- Venkatesh, A., & Prakash, V. (1993). Functional properties of the total proteins of sunflower (*Helianthus annuus* L.) seed: effect of physical and chemical treatments. *Journal of Agricultural and Food Chemistry*, 41, 18–23.
- Zhong, Z. K., & Sun, X. S. (2000). Thermal behavior and nonfreezing water of soybean protein components. *Cereal Chemistry*, 77(4), 495–500.