Effect of thermal processing on protein solubility of green gram (*Phaseolus aureus*) legume cultivars

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Abstract Green gram legume cultivars were analyzed for their protein solubility profile by fractionation in the raw form and also after heat processing. The results indicated that globulin fractions, which are present in major amounts that ranged from 79.5 to 85.4 % significantly decreased after the heat treatment. This decrease was accompanied by a significant increase in the glutelin-3 fractions. The prolamine contents did not vary considerably after processing. The protein and non-protein nitrogen contents ranged from 22.6 to 26.2 % and 2.3 to 2.7 % in the legume cultivars, respectively. The antinutritional factors like total polyphenol and phytic phosphorous were also determined. The accumulation of polyphenols was in the seed coat portion of the legume where as that of phytic phosphorus was in the cotyledons. SDS — PAGE profiles of all the three green gram cultivars had five major polypeptides (molecular weight 15, 18, 20, 45 and 60 kDa) in the total protein composition. Wide variation in electrophoresis pattern was observed after heat processing. Thermal treatment increased the insoluble protein fractions and eliminated the minor polypeptide bands below 14.3 kDa in the green gram cultivars.

Keywords Cooking · Green gram · Protein solubility · Globulins · Albumins · Prolamines

Introduction

Green gram (*Phaseolus aureus*)/mung bean is one of the widely used legumes of India, to meet the protein requirements. The mung bean has several botanical synonyms including *Azuki*

radiata (L) Ohwi, *Phaseolus aureus* (Roxb.) Maekawa, *Vigna aureus* (Roxb) Hepper and *Vigna radiata* (L.) R Wilczek (Nowkolo and Smartt 1996). The crop is said to have originated in India and must have been derived from var. *sublobata* which occurs wild throughout India and Burma (Aykroyd and Doughty 1964; Purseglove 1977). It is consumed in India and in many other Asian countries as a vegetable in the form of cooked beans or as mung bean sprouts. Mung bean contains 20–25 % protein and sufficient quantities of all amino acids except methionine, cystine and tryptophan (Evans and Bandemer 1967). This legume is generally consumed after suitable processing like soaking, cooking, popping, grinding etc. in India and widely used for the preparation of a number of products, including various sweets and snacks. The grains after dehulling are generally known as “dhals” (dehulled split cotyledons) in India. The importance of legumes as components of traditional diets worldwide is based on their high protein, starch and lysine contents (Clark and Switzo 1975). Good growth response was recorded when mung bean formulations were given to children in Thailand and the Philippines (Bhumiratana and Nondasuta 1969; Alcaraz-Bayan et al. 1972).

It has been suggested that the improvement in the protein digestibility of different legumes may be attributed to inactivation of trypsin inhibitors during heat treatment (Khokhar and Chauhan 1986; Salunkhe and Kadam 1989). Thermal aggregation (or coagulation) and gelation are important functional properties of food proteins, contributing to mouth feel and texture of many food systems. Denaturation is a process in which proteins undergo conformational changes, primarily unfolding, without alteration of the amino acid sequence (Hermansson 1979). He defined that coagulation is the random interaction of protein molecules, leading to formation of aggregates that could be either soluble or insoluble. Thus for proteins, this property assumes importance in its utilization.

The basic reason for heating a food product is to make it palatable and safe to consume or to prevent or minimize spoilage during storage and these processing can give rise to

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major changes in composition. Solubility of protein is considered as the most important factor and an excellent index for their functionality of dehydrated products. In addition it is an important factor because of its relevance to other properties such as viscosity, gelation, foaming and emulsification. The method of processing affects the solubility of protein especially if they are exposed to heat (Kilara and Harwalkar 1996). In scanning electron microscopic studies of cooked spaghetti and cooked noodle structure it is suggested that the manner in which the proteins were modified during cooking process might account for cooking quality differences (Dexter et al. 1978; Dexter and Dronzek 1979). Nowadays, there is an increasing preference for vegetarian diets and many industries are diversifying their range of legume based products. Thus current study aims to decipher the protein solubility pattern of green gram cultivars due to heat processing, their resultant modification in structure by partial or complete denaturation shown by SDS PAGE which helps to understand their importance for better nutritive utilization. Also, information on the distribution of seed protein fractions of green gram is limited and hence this study.

Materials and methods

Seed material

Green gram cultivars namely KOP and PKV were purchased from Punjab Rao Krishi Vidya Peeth (PKV), Akola, Maharashtra, India and another variety was purchased from the local market. The grains were cleaned and freed from foreign materials such as chaff and stones. A portion of the seeds was dehulled in the CFTRI designed versatile dhal mill as per the method of Kurien (1984). All the materials including whole legume, dehulled splits (*dhals*) and the seed coats were stored in cold room and were drawn for the experiments as and when required and used after allowing them to equilibrate at room temperature for 24 h. The whole seeds were pulverized in a laboratory coffee grinder to pass through 250-micron sieve and stored in airtight plastic containers and used for the analysis.

Color measurement

The color measurement of the green gram cultivars was done using Shimadzu UV visible spectrophotometer UV-2100 model attached to Shimadzu compartment — MPC 3100 by CIE method. The color values are expressed as ‘L*’ (whiteness or darkness), ‘a*’ (redness/greenness) and ‘b*’ (blueness/yellowness) values. The instrument was calibrated to the white using white beads ($L^*=99.87, a^*=-0.06, b^*=0.37$) prior to each usage. The measurements were done using 100 g seeds/dhals tightly packed in transparent polyethylene bags

such that light could not pass freely through it. Color was measured in triplicate and the average values reported.

Thermal processing

About 25 g of whole seeds were added to 250 ml of boiling distilled water in a beaker kept on a heater. Grains were drawn at various intervals and pressed between thumb and forefinger to check for the hardness. When the grains yielded to minimum pressure, they were adjudged as cooked grains. Then after cooling to room temperature, the cooked grains along with cook water were homogenized in a blender and later freeze dried to obtain moisture of ~7 % and used for further analysis.

Total nitrogen

Total nitrogen in the samples was determined by microkjeldahl method as described in AOAC (1995) and the crude protein was calculated using a factor of 6.25.

Non protein nitrogen

The non-protein nitrogen content of the samples was determined by AOAC Official methods of Analysis (1984).

Total polyphenols

The total polyphenols were determined as tannic acid by method described by Yen and Hsieh (1998). One gram sample was extracted with 50 mL of 0.1 M HCl in methanol–water (60:40, v/v). One hundred microlitres of phenolic extract was added to 2 mL of aqueous sodium carbonate solution (20 g/L). After 2 min, 100 μ L of Folin-Ciocalteu reagent (1:1) was added to the mixture and mixed thoroughly. The absorbance was measured at 750 nm after 30 min against distilled water. A calibration curve for tannic acid (20–100 μ g) was used and the phenolic compounds were expressed as tannic acid.

Phytic acid

The phytic acid content was determined by the method of Haug and Lantzsch (1983). 100 mg powdered sample was extracted with 5 ml of 0.2N HCl into a test tube with a ground glass stopper. To it, 1 ml of 0.2 % ammonium iron (III) sulphate solution prepared in 2N HCl and made up to 1,000 ml with distilled water was added. The test tubes were covered with stoppers and fixed with clips. The tubes were heated in boiling water bath for 30 min. Care was taken to see that the tubes were well stoppered for the first 5 min. After cooling in ice water for 15 min, allowed to adjust to room temperature. Added 2 ml of 2,2' bi pyridine solution (2.5 g dissolved in 2.5 ml of thioglycollic acid and made up to

250 ml with distilled water), mixed the contents and measured the absorbance against distilled water at 519 nm. The phytic acid content was calculated from a calibration curve using phytate phosphorus salt in the range of 3–30 µg.

Separation of protein fractions

The nitrogen from defatted meal was extracted stepwise by a series of solvents according to procedure of Landry and Moureaux (1970). Defatted samples weighing 3.5 g (db) were suspended in 35 ml of 0.5 M NaCl and kept for magnetic stirring at 4 °C, for different periods of 60, 30 and 30 min (i.e. three intervals of separate extractions) respectively. They were centrifuged at 3000g for 15 min and the filtrates combined to get the globulin fraction. The residue was further extracted as above using water for 15 min each twice and extracts combined after centrifugation to obtain albumin fraction. The residue was then treated with 60 % ethanol for 30 min twice at 20 °C and then with 55 % iso-propanol at the same temperature for 60, 30 and 15 min respectively. The centrifugates were combined to get prolamine fraction. The pellet was further treated twice with 60 % ethanol containing 0.6 % 2-mercaptoethanol for 30 min followed by 55 % isopropanol with 0.6 % 2-mercaptoethanol for 30 min twice at 20 °C in order to get G1 type glutelins. Then the residue was extracted with borate buffer (pH 10) with 0.6 % 2-mercaptoethanol and 0.5%NaCl for periods of 60, 30 and 30 min each at 20 °C. The centrifugates were combined to get G2 type glutelins. Lastly, the residue obtained after centrifugation was extracted with borate buffer along with 0.6 % 2-mercaptoethanol and 0.5 % Sodium dodecyl sulphate for 60,30 and 30 min at 20 °C to get G3 type glutelins. For each solvent, supernatants were combined to give total extract. The nitrogen content of each of these six fractions was determined by the micro- Kjeldahl method. The residue left after extraction was also analyzed for nitrogen content.

SDS-PAGE

SDS-PAGE of protein samples was carried out by the method of Laemmli (1970). The discontinuous system used consisted of a 5 % (w/v) acrylamide stacking gel and a 12.5 % (w/v) acrylamide separating gel. Samples (1 mg/ml protein) were dissolved in tris/glycine (pH 8.9) containing 20 g/l SDS and

50 g/l 2-mercaptoethanol. Electrophoresis (Bangalore Genei Pvt Ltd, Bangalore, India) was carried out at constant voltage (50 V) until the tracker dye reached the bottom of each gel. After electrophoresis, the gels were stained with 0.2 % (w/v) Coomassie brilliant blue R-250 (in 10 % (v/v) acetic acid: 50 % (v/v) methanol) and destained with 10 % (v/v) acetic acid containing 40 % (v/v) methanol for 16 h. Protein markers used were phosphorylase b (97.4 kDa), bovine serum albumin (66 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa), soybean trypsin inhibitor (20.0 kDa) and Lysozyme (14.3 kDa) (Bangalore Genei, Bangalore, India).

Statistical analysis

All results in this study are reported as means of three replicate analyses. One-way analysis of variance (ANOVA) was carried out to compare the mean values followed by Duncan's multiple-range test (Duncan 1955).

Results and discussion

Analyses of protein and non protein nitrogen

The total protein content of the green gram cultivars in the whole seed form ranged from 22.6 to 26.2 % (Table 1). The seed coat fractions had a total protein content of 6.9 to 8.4 %, the highest being the PKV variety. The total protein content is affected by several parameters including soil type, climatic conditions, region, use of fertilizers, and genetic factors (Mosse and Pernollet 1982; Deshpande and Damodaran 1990). Legume proteins consist of storage and metabolic proteins. Storage proteins usually account for the largest portion of the total proteins and may constitute up to 80–90 % of the total proteins.

The non protein nitrogen content (NPN) among the three green gram cultivars are presented in Table 1 which ranged between 2.0 % and 2.7 % in whole form, 2.2 to 2.6 % in dhal form and 1.5 to 2.1 % in the seed coat fractions. While expressing as the percent of the total protein content of respective forms of the seed like whole, dehusked and seed coat, the latter had highest percentage of non-protein nitrogen content (22 % to 28 %) (Fig. 1). Earle and Jones (1962) have carried out the quantitative analysis of NPN in many legumes.

Table 1 The total Nitrogen ($N \times 6.25$) and Non Protein Nitrogen ($N \times 6.25$) (NPN) in cultivars of Green gram legume cultivars, dhals and seed coats

Cultivars	Whole		Dhals		Seed coat	
	Total N ₂ (%)	NPN (%)	Total N ₂ (%)	NPN (%)	Total N ₂ (%)	NPN (%)
KOP	26.25±0.48a	2.33±0.04b	25.00±1.32a	2.60±0.14b	6.95±0.29a	1.55±0.06a
PKV	22.60±1.18a	2.00±0.10a	24.90±0.25a	2.24±0.02a	8.42±0.44b	2.08±0.11b
Local	24.15±0.33a	2.74±0.04c	26.88±1.43a	2.42±0.13ab	7.57±0.86ab	2.13±0.24b

Mean scores in a column with different letters are significantly different at $p < 0.05$

Fig. 1 Relative non-protein nitrogen content in green gram cultivars

Many dry beans contain 8–15 % nitrogen as non-protein nitrogen which includes free amino acids, pyridines, pyrimidines, nucleic acids, alkaloids, amines and complex lipids; the protein content may be overestimated by 1–2 % on a seed weight basis (Earle and Jones 1962; Deshpande and Nielson 1987). The ratio of NPN to total seed nitrogen is in the range of 10–15 %. Among commonly cultivated legumes, NPN was reported to be maximum in moth bean and horse gram constituting 12 to 19.6 % of total nitrogen (Gupta 1983). Evans and Bandemer 1967 have observed that the non-protein amino acid, S-methylcystine is present in mung bean but has not been linked to any harmful effects.

Protein fractionation

Based on the solubility classification, legume storage proteins are primarily globulins (soluble in dilute salt solutions) followed by the albumins (water soluble proteins). Legume protein quality is attributable to the higher levels of the albumin and globulin fractions, which are rich in the essential amino acid lysine. Table 2 shows the variation in protein fractions in green gram cultivars before and after thermal

processing. The total protein content in the raw pulse ranged from 22.6 to 26.2 % while it was 22.3 to 25.1 % after thermal processing. Table 2 data also indicate that the green gram cultivars contained high amounts of salt soluble globulin fractions (79–85 %) of the total protein. Similarly, major portion of beans was in the form of globulins in all the three cultivars of green gram (Fig. 2). Nikokoyris and Kandylis (1997) too have reported that in faba bean, using similar extraction procedure, a major portion of globulins followed by glutelins and lesser amounts of albumins and prolamines were obtained. Vasconcelos et al. (2010) in their studies with cultivars of cowpea have reported globulins as the major fraction after protein fractionation. Albumins are known to be rich in sulphur-amino acid and other EAA (Baudoin and Maquet 1999).

Heat treatment for optimum periods has been reported to improve the protein quality of legumes by improving protein digestibility and protein efficiency ratio. Heating also inactivates trypsin and amylase inhibitors. Heat treatment can also produce acceptable flavours and colours in food legumes. However extended cooking at high temperatures and pressures lowers nutritional quality of legumes (Uzogara et al.

Table 2 Changes in protein fractions of raw and cooked seeds of green gram cultivars

Cultivars	KOP		PKV		Local	
	Raw	Cooked	Raw	Cooked	Raw	Cooked
Seed protein* (g/100 g dry matter)	26.25c	25.13bc	22.60a	22.30a	24.15b	23.74ab
Globulins	85.45d	15.80b	79.56c	8.30a	80.58c	14.66b
Albumins	1.03a	1.47b	1.77c	2.05c	2.48d	1.22ab
Prolamins	3.70a	4.54b	5.51c	5.32c	4.97bc	5.43c
G1 glutelins	2.06c	2.67d	1.51c	1.93bc	1.70ab	1.98bc
G2 glutelins	4.61b	21.25d	1.32a	8.65c	1.95a	3.58b
G3 glutelins	3.66a	54.52c	9.16b	70.67d	7.16b	68.66d
Insoluble protein	0.72a	1.95b	1.99b	5.38d	2.82c	6.28e
Total protein recovered (%)	101.24a	102.21a	100.83a	102.31a	101.67a	101.80a

Mean scores in a row with different letters are significantly different at $p < 0.05$

* $N \times 6.25$

Fig. 2 Pattern of protein fractions of green gram whole legume cultivars (raw and cooked). *G* Globulins, *A* Albumin, *P* Prolamine, *G1*, *G2*, *G3* Glutelins, *IP* Insoluble proteins

1992). The desirable effects of heating legumes can be attributed to denaturation of globulin, which is normally resistant to denaturation and digestion in the native state. The initial rise in PER has been reported to be due to rapid inactivation of the proteinaceous antinutrients (Bressani 1985).

When proteins of cooked pulses were fractionated, it was found that the globulin fractions reduced significantly in all the three cultivars (66–71 %) (Table 2), (Fig. 2). Semino and Cerletti (1987) reported that heat treatment degraded the high molecular weight polypeptides, giving rise to smaller fragments. The results obtained in this study are in agreement to those reported by Nugdallah and El Tinay (1997) who indicated that the globulin and albumin fractions of cowpea decreased significantly ($p \leq 0.05$) as a result of cooking. Albumin fractions of raw green gram cultivars ranged from 1.03 to 2.48 % of the total protein (Table 2). However, the albumin fractions in the two pulse cultivars (KOP and PKV) showed marginal increase (0.28–0.44 %) after cooking while there was 1.26 % decrease in local cultivar (Fig. 2). Heating is responsible for protein denaturation, eventually followed by aggregation of the unfolded molecules, which results in loss of solubility. Thermal denaturation involves an initial stepwise dissociation of subunits and a subsequent re association of only partially unfolded molecules with formation of either soluble or insoluble complexes (Kinsella et al. 1985).

The prolamine fractions of the cooked green gram cultivars ranged from 4.5 to 5.4 % compared to 3.7 to 5.5 % for the raw green gram (Table 2). Thus an increase in prolamine content was observed as a result of cooking except in PKV cultivar which indicated a small decrease of 0.19 %. G1 glutelin fractions indicated minimum increase from (0.28 to 0.61 %) after cooking, where as G2 fraction increased from 1.63 to 16.64 % range. However, maximum increase (50–61 %) was noticed in glutelin-3 like fraction of cooked green gram (Fig. 2). Similar observation has been made by Hala et al. (2003) who studied the effect of cooking on protein solubility profiles of faba bean under two water regimes. Heating a protein at 100 °C in sodium dodecyl sulphate (SDS) solution (usually in the presence of thiols) will completely dissociate all polypeptide chains from one another (Clark and Switcho 1975). This increases the low molecular weight fractions in the heat treated samples and results in decrease in the high molecular weight fractions through cleavage of disulphide linkages. The unfolding of protein molecules will increase accessibility to digestive enzymes thereby enhancing protein digestibility. The insoluble protein (residue) consists mainly of proteins from previously defined groups, becoming insoluble due to interactions with lipids, carbohydrates, or polyphenols via oxidation (Landry and Moureaux 1981). The protein fractionation revealed that the globulin fraction reduced considerably in all the three cultivars after cooking, with an increase in the glutelin-3 fraction (Fig. 3).

Fig. 3 Percent increase or decrease of various protein fractions after cooking.
G Globulins, *A* Albumin, *P* Prolamine, *G1*, *G2*, *G3* Glutelins, *IP* Insoluble proteins

SDS PAGE

Electrophoresis patterns of raw and cooked green gram cultivars are shown in Fig. 4. In this procedure the same amount of protein (60 µg) from each sample was loaded into the gel wells to allow a better comparison. Protein profiles obtained from native samples resembled each other both qualitatively and quantitatively. The pattern indicates that they possess proteins with wide range of molecular weights. SDS — PAGE profiles of all the three green gram cultivars had five major polypeptides (molecular weight 15, 18, 20, 45 and 60 kDa) in the total protein composition. A large number of polypeptide chains with molecular weight range of 25–90 kDa were detected in all the three green gram cultivars. There were also low molecular weight polypeptides in the range of 14.3 to 25 kDa. Green gram contains both legumin and vicilin of which the latter is more abundant. Legumin is composed of three polypeptides of MWs 37,000, 34,000 and 20,000 (Derbyshire and Boulter 1976) whereas vicilin (8S) contains

Fig. 4 SDS PAGE patterns of protein fractions of green gram raw and cooked. *1a* KOP Raw, *1b* KOP cooked, *2a* PKV Raw, *2b* PKV cooked, *3a* Local Raw, *3b* Local cooked, *4* Molecular weight markers

four sub units with MWs 63,000, 50,000, 29,000 and 24,000. The electrophoretic patterns of seeds protein fractions for all three green gram cultivars resembled to each other qualitatively. I.M. Vasconcelos et al. (2010) in their studies on cowpea cultivars too have indicated similar electrophoretic patterns in three cowpea cultivars.

The intensity of both high and low molecular weight polypeptides decreased upon cooking. However, the most noticeable observation in the protein profile was the absence of low molecular weight polypeptides in the cooked samples in the molecular weight below 14.3 kDa. The total protein electrophoretic profiles of green gram resemble the protein profiles of *Dolichos lab lab*, where as many as 16 polypeptides in a seed total protein profiles with a molecular mass range of 24–60 kDa and a few more polypeptides in molecular mass range 14.3–24 kDa have been reported (Shastry and John 1991). Thus SDS PAGE of raw and cooked green gram cultivars revealed that the intensity of both high and low molecular weight polypeptides decreased upon cooking. Meng et al. (2002) too had reported that red bean globulins are heat sensitive. Thus the thermal treatments affected the protein solubility profile.

Polyphenols and Color

The total polyphenols of green gram cultivars, in the whole pulse form, dehulled cotyledon (dhals) and seed coat are presented in Table 3. The difference in polyphenol content was more pronounced in the seed coats in all three cultivars. The whole seeds had a polyphenol content of 4.55 to 5.00 mg/g, which reduced considerably after dehulling. The percent reduction of polyphenols after dehulling was in the range of 71–80 % among the three cultivars. The seed coat had maximum amount of polyphenols that ranged from 9.4 to

Table 3 Total polyphenols and Phytic phosphorous content in components of green gram cultivars

Cultivars	Polyphenols (mg/g)			Phytic acid ($\mu\text{g}/100\text{ mg}$)		
	Whole	Dhals	Seed coat	Whole	Dhals	Seed coat
KOP	4.55a	1.30a	152.80b	39.24b	60.32b	2.80a
PKV	5.00a	1.04a	99.05a	40.89b	60.26b	2.81a
Local Gg	5.05a	1.18a	94.05a	19.72a	42.09a	2.81a

Mean scores in a column with different letters are significantly different at $p < 0.05$

15.28 mg/g. Among the three cultivars, the cultivar KOP of green gram had darker seed coat color and showed higher polyphenols content (15.28 mg/g). Phenolic compounds are common in plants including legumes. Cotyledon fractions contain very low concentrations of phenolics. In our previous studies (Sreerama et al. 2010), we have obtained similar results for chickpea and horse gram cotyledons, although the levels of phenolic compounds were much lower (15.24 mg, 13.81 mg of GA/g respectively). The phenolic compounds mostly concentrated in the seed coat fractions and could be easily removed by dehulling. The phenolic compounds content in cotyledon fractions of green gram cultivars was lower than those reported for beach pea (Shahidi et al. 2001). Plant phenolics include several compounds of which tannins are important. In the recent years, polyphenols in many edible plant products have received increasing attention as a result of their influence on the nutritional and aesthetic quality of foods, biochemical and physiological functions and pharmacological implications (Goodwin and Mercer 1983).

Within the green gram cultivars, the KOP cultivar had uniform colored grains (Table 4). Non-uniform size and shape of the samples may contribute to the variation of the color readings. The color parameter L^* which represents lightness was in the range 45 to 46 for whole grains and 69 to 73 for the dehulled splits indicating its lighter color. Regarding the ' a^* ' value which denotes the redness of the sample and the yellowness value ' b^* ' was high in split dehulled cotyledons

Table 4 Instrumental color parameters of green gram legume cultivars and their dhals/Dehulled splits

Seed form	Cultivar	L^*	a^*	b^*
Whole legume	KOP	46.03a	1.73a	11.00ab
	PKV	45.55a	1.86a	11.89b
	Local	45.90a	1.93a	9.73a
Dhals/Dehulled splits	KOP	73.72c	7.97c	29.16d
	PKV	73.80c	6.17b	23.28c
	Local	69.75b	8.62c	27.66d

Mean scores in a column with different letters are significantly different at $p < 0.05$

(dhals) as compared to the whole grains. The ' a^* ' values for whole grains and dhals ranged from 1.7 to 1.9 and 6.1 to 8.6 respectively. The ' b^* ' values were 9.7 to 11.9 and 23.2 to 29.1 for whole grains and dhals respectively. The majority of grains are opaque but not optically homogeneous. Surface characteristics varying from smooth to rough and from flat to curve can also affect the instrumental color readings.

Kachare et al. (1988) showed that seed color was associated with content of polyphenols. However, in our study of green gram cultivars, the seed coat colour could not be corroborated to polyphenols content. Cowpea cultivars with colored seed coats contain larger amounts of polyphenols than those with white or cream colored seeds. Similar observation was made by Ologhobo and Fetuga (1984) also. Reddy et al. (1985) have reported the tannin content of common beans in the range of 0.0 to 2.0 %, depending on the bean species and color of the seed coat. They have also observed that condensed tannins constituted the major portion of the common bean tannin content, which are more widely distributed among higher plants. Dehulling removed most of the tannins from the colored grains. Bressani and Elias (1980), Deshpande et al. (1982) have also stated that major amounts of bean tannins are located in the seed coat with low or negligible amounts in the cotyledons. Thus the dehulled seeds of all the cultivars investigated did not differ significantly. Tannins are associated mainly with seed coats of legumes, whose tannin content varies considerably (78 to 1,710 mg/100 g) between different legumes and cultivars. Cooking the seeds reduces their tannin content by about 70 % (Bressani and Elias 1980; Rao and Deosthale 1982). However, its presence is also beneficial because of its positive roles as a bioactive component.

Phytic acid

The phytic acid content in green gram whole grain, dhal and husk is given in Table 3. It varied from 19 to 40 $\mu\text{g}/100\text{ mg}$ in green gram whole grain, 42 to 60 $\mu\text{g}/100\text{ mg}$ in dhals and 2.80–2.81 $\mu\text{g}/100\text{ mg}$ in the seed coats. It can be seen that the phytates are concentrated in the cotyledon portion of the seeds in the legume. We have observed in our previous studies that seed coat fractions showed lowest phytic acid content, 0.79 mg/g in chickpea and 1.02 mg/g in horse gram (Sreerama et al. 2010). Kakati et al. (2010) have reported much higher values of phytic acid content (664.76 to 692.4 mg/100 g) in two newly developed cultivars namely SHC16 and SHC 20 and presumed that the variation of phytic acid content in different cultivars could be due to the differential accumulation depending on genetic control of their synthesis that influences deposition. Pande et al. (2012) in their studies using microwave drying of green gram reported a value of 591.79 mg/100 g of phytic acid. These differences could probably due to varietal, agro climatic or genetic variations, combined with change in methodologies and procedures that exist in various laboratories.

Reddy et al. (1982) have reported that in dicotyledonous seeds, including legumes and oilseeds, phytates are distributed throughout the cotyledon and located within the subcellular inclusions of aleurone grains or protein bodies. Elkowicz and Sosulski (1982) have observed that phytic acid is found in mung bean in amounts much lower than those reported for soybean and navy bean. Phytic acid and its salts (hexaphosphate myoinositol) are the principal reserve of phosphate of plants. It has the capacity to form insoluble complexes with polyvalent cations like, copper, zinc, cobalt, manganese, iron and calcium which precipitate in the intestinal lumen thus becoming unavailable to the organism. They can also form complexes with the proteins that are very resistant to proteolytic digestion; reducing the already low digestibility of the legume proteins. It is quite stable in thermal treatments, undergoing only partial hydrolysis (Cheryan 1980; Maga 1982).

Conclusions

The three green gram legume cultivars in native form did not differ much in their protein content, fractions or the electrophoresis profile. The protein solubility profiles showed variation in different extractants after thermal processing, and also the intensity of bands in electrophoresis reduced. All the cultivars had higher amount of globulins as the major fraction, which is the storage protein of legumes and contains the essential amino acid lysine. The polyphenols were concentrated in the seed coat portion while phytic phosphorous was distributed in the cotyledon portion of green gram which offers a choice depending on the end product. Thus processing of legumes assumes importance in the overall quality profile of legumes which is an important criterion to use them as functional foods. Hence thermal processing is one of the best ways to avail the benefits of nutritive value.

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