Chapter 2
# Chapter 2

Probing novel allergenic proteins of commonly consumed legume using Simulated Gastric Fluid (SGF) assay

## 2.1. Introduction

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2.1. Introduction

Resistance to digestion has been shown to be a characteristic feature of several food allergens (Astwood et al., 1996; Besler et al., 2001). This characteristic of allergenic proteins is mostly tested by analyzing the effects of simulated gastric fluid (SGF) treatment on the integrity of proteins. Furthermore, because of this property of allergenic proteins, resistance to pepsin digestion has been incorporated in the FAO/WHO decision tree approach (2001) and Codex Alimentarius Guidelines (2003) for the allergenicity assessment of genetically modified (GM) foods. Stability of proteins to SGF is usually evaluated by means of SDS-PAGE fractionation, followed by protein staining.

Pepsin resistance of allergens imparts an increased probability of reaching it to intestinal mucosa, where it could be exposed to intestinal immune system, resulting in allergic/immune response. A number of food allergens have been reported to be stable in SGF. Astwood et al., (1996) compared the digestive stability particularly with reference to purified allergenic and non-allergenic food proteins in standard SGF and inferred that most of the non-allergenic proteins degrade within 15 seconds without forming any stable fragments, while some food allergens or parts of it were stable in SGF even up to 60 minutes. A number of other studies support a correlation between non-digestibility of a protein in SGF to its allergenicity (Bannon et al., 2003; Koppelman et al., 2005).

It was thought that SGF assay may be used as a tool to screen pepsin resistant proteins of different leguminous crops in their crude protein extract (CPE). Therefore, objective of this study was to screen pepsin resistant and subsequently probable allergenic proteins of different leguminous crops namely, chickpea, green gram, red gram, black gram, kidney bean and
bengal gram that are consumed mainly in India and neighboring countries. Soybean and peanut CPE were taken as positive control, where characteristic details of allergens including their molecular weights (mol wts) were known.

2.2. Materials and methods

2.2.1. Chemicals

Pepsin from porcine gastric mucosa (Catalog number P 6887), purified proteins like, β-amylase from sweet potato, lipoxygenase from soybean (Type I-B, lyophilized), ovalbumin from chicken egg white, lysozyme from chicken egg white, β-lactoglobulin B from bovine milk, acrylamide, N,N' methylene-bis-acrylamide, sodium dodecyl sulphate, ammonium per sulphate, N,N,N',N'-tetramethylethylenediamine, coommasie brilliant blue (CBBR-250) and wide range (205-6.5 kDa) molecular weight markers were purchased from Sigma Chemical Company (St. Louis, MO, USA). Trichloroacetic acid (TCA) and Folin-Ciocalteu's reagent were procured from BDH and Qualigen Fine Chemicals, Bombay, India. All other chemicals were of analytical grade of purity and procured locally.

2.2.2. Reagents

Protein extraction Phosphate buffer

20 mM Na₂HPO₄
2 mM KH₂PO₄
5.4 mM KCl
0.5 M NaCl (pH 7.0)

Stopping Solution (2X)

200 mM Dithiothreitol
4% SDS
0.2% Bromo Phenol Blue
20% glycerol
100 mM Tris (pH 8.8)
2.2.3. Collection of legume seeds

Dry legume seeds such as *Cajanus cajan* (Pigeon pea, Red gram, commonly known as Arhar), *Phaseolus aureus* (Green gram, commonly called as Mung), *Phaseolus mungo* (Black gram, commonly known as Urad), two varieties of *Cicer arietinum* [cultivars i.e. Kabuli type: chickpea (commonly known as, chhole) and desi type (also known as Bengal gram)], *Phaseolus vulgaris* (Kidney bean, commonly called as Rajma), *Arachis hypogea* (peanut) and *Glycine max* (soybean) were procured from local market.

2.2.4. Preparation of Crude Protein Extracts (CPEs)

Proteins from different crops were extracted as described by Astwood *et al.* (1996) with slight modifications. Briefly, 25 gm seeds were powdered using a dry grinder and defatted with n-hexane. Defatted flour was macerated for 2 minutes in grinder along with the protein extraction phosphate buffer. The mixture was agitated overnight at 4 °C and centrifuged for 30 min at 10,000g. The supernatant was recovered, filtered through 0.45 μm syringe filter and stored at -80 °C until used.

2.2.5. Protein Estimation

The protein content in TCA precipitate was estimated according to the method of Lowry *et al.*, (1951), using bovine serum albumin (BSA) as standard. Precipitated protein was dissolved in 1.0 ml of 1 N sodium hydroxide and diluted to 10.0 ml with distilled water. To 1.0 ml of aliquot, 5.0 ml of alkaline copper reagent (98 ml of 2% sodium carbonate in 0.1 N sodium hydroxide, 1.0 ml of 0.5% copper sulphate and 1 ml of 1% sodium potassium tartrate) was added and the solution was kept at room temperature for 10 minutes. Colour was developed by the addition of 0.5 ml of 1 N Folin-Ciocalteau reagent and the intensity of blue colour was
measured after 30 minute at 660 nm. The result was expressed as mg protein/ml.

2.2.6. Preparation of SGF

SGF was prepared as described by United State Pharmacopoeia (1995). Pepsin, 3.2 mg (approximately 3460 units activity/mg) was dissolved into 1 ml of 34 mM NaCl and 0.7% HCl, pH 1.2. Activity of each newly prepared SGF solution was defined as the production of a Δ A280 of 0.001 per min at pH 1.2 at 37 °C measured as TCA soluble products with hemoglobin as substrate. SGF solution was freshly prepared and used within the same day.

2.2.7. Digestibility of purified food proteins and CPEs in SGF and SDS-PAGE analysis

SGF assay was performed taking purified non-allergenic proteins (β-amylase and lipoxygenase) and allergenic proteins (ovalbumin, lysozyme and β-lactoglobulin). The digestibility of all these proteins was examined as described by Roesler and Rao (2001) with minor modifications. All proteins were dissolved at a concentration of 1 mg/ml in deionized water. SGF (430 μl) was incubated at 37 °C prior to addition of 86 μl of test protein solution. The contents of the tube were mixed by mild vortexing and the tubes were immediately placed in a 37 °C water bath. The reaction was stopped at different time intervals (0.25, 0.5, 1, 2, 4, 8, 15 and 60 minutes) by the addition of 60 μl of the incubation mixture to 60 μl of stopping solution. SGF and protein were added directly to the stopping solution prior to the incubation, for zero min control. Each sample was boiled for 5 min at 100 °C. Boiled samples (15 μl/well) were subjected to SDS-PAGE according to the method of Laemmli (1970), using 15 well, 1 mm thick, 14% polyacrylamide midi gels of 18 x 16 cm (Amersham Biosciences, San Francisco, USA) at 22 mA for 4:30 h. Standard molecular weight markers were included in all gels to estimate
the mol wt of the proteins. Coommasie brilliant blue (CBB) (R-250) staining was employed for all the gels. Gel images were captured using Syngene Bio Imaging Systems (Syngene, Cambridge, UK).

Digestibility of CPE of soybean, peanut, chickpea, black gram, kidney bean, Bengal gram, red gram and green gram was performed essentially as described above. The only difference was in starting protein concentration of various CPE prior to SGF digestion (4 mg/ml instead of 1 mg/ml).

2.2.8. Densitometry analysis of undigested proteins
The resulting undigested bands were quantified using the Genetools Software version 1.02 (a) (Syngene, Cambridge, UK) where raw volume of the bands was recorded. The raw volume was defined as a three-dimensional size of the band where intensity was the third dimension. Each sample protein was digested at least 4 times and was subjected to reproducible SDS-PAGE. Densitometry of each protein band was performed thrice. The average values have been used for preparing graphs.

2.3. Results
2.3.1. Digestibility of non-allergenic and allergenic purified food proteins in SGF
Stability in SGF was defined as the last time point for which the protein or its proteolytic fragments could be detected by SDS-PAGE and CBB staining. Two food proteins taken as non-allergenic representative namely, β-amylase and lipoxygenase were completely digested within 15 seconds in the SGF (Fig. 2.1 and 2.2). In contrast, the stability of representative food allergenic proteins; lysozyme, ovalbumin and β-lactoglobulinm was 8 min, 15 min and 60 min, respectively (Fig. 2.3-2.5).
Fig. 2.1. (a) SDS-PAGE analysis of SGF digestion of β-amylase.
Lane 1: Pepsin, Lane 2: β-amylase, Lane 3: 0 min,
Lane 4-11: SGF digestion pattern of β-amylase at time points: 0.25, 0.5, 1, 2, 4, 8, 15 and 60 min,
Lane M: Molecular weight markers.
(b) Quantification of density of β-amylase.
Values are means of triplicate analysis.
Fig. 2.2. (a) SDS-PAGE analysis of SGF digestion of lipoxygenase. Lane 1: Pepsin, Lane 2: lipoxygenase, Lane 3: 0 min, Lane 4-11: SGF digestion pattern of lipoxygenase at time points: 0.25, 0.5, 1, 2, 4, 8, 15 and 60 min, Lane M: Molecular weight markers.
(b) Quantification of density of lipoxygenase. Values are means of triplicate analysis.
Fig. 2.3. (a) SDS-PAGE analysis of SGF digestion of lysozyme. 
Lane 1: Pepsin, Lane 2: lysozyme, Lane 3: 0 min, 
Lane 4-11: SGF digestion pattern of lysozyme at time 
points: 0.25, 0.5, 1, 2, 4, 8, 15 and 60 min, 
Lane M: Molecular weight markers.
(b) Quantification of density of lysozyme. 
Values are means of triplicate analysis.
Fig. 2.4. (a) SDS-PAGE analysis of SGF digestion of Ovalbumin. Lane 1: Pepsin, Lane 2: Ovalbumin, Lane 3: 0 min, Lane 4-11: SGF digestion pattern of Ovalbumin at time points: 0.25, 0.5, 1, 2, 4, 8, 15 and 60 min, Lane M: Molecular weight markers. (b) Quantification of density of Ovalbumin. Values are means of triplicate analysis.
Fig. 2.5. (a) SDS-PAGE analysis of SGF digestion of β-lactoglobulin. Lane 1: Pepsin, Lane 2: β-lactoglobulin, Lane 3: 0 min, Lane 4-11: SGF digestion pattern of β-lactoglobulin at time points: 0.25, 0.5, 1, 2, 4, 8, 15 and 60 min, Lane M: Molecular weight markers. (b) Quantification of density of β-lactoglobulin. Values are means of triplicate analysis.
2.3.2. Digestibility of CPE in SGF

Fig. 2.6 a shows the digestion pattern of CPE of soybean in SGF. SDS-PAGE of soybean CPE before SGF digestion (Fig. 2.6, lane 2) presented 10 distinct proteins with apparent mol wts ranging from 6.5 to 97 kDa. As estimated from standard mol wts marker (Fig. 2.6, lane M), four proteins of approximately 65, 22, 20 and 6.5 kDa persisted up to 60 min (Fig. 2.6, lane 11), while 26 and 70 kDa proteins remained stable till 8 and 2 min, respectively (Fig. 2.6, lane 9 and 7). Remaining four proteins of mol wts of approximately 97, 84, 80 and 29 kDa were rapidly digested in SGF in less than 15 sec. Densitometric analysis of the undigested proteins of soybean CPE after SGF digestion at different time intervals was performed to know relative amount and its stability towards pepsin (Fig. 2.6 b). Protein of 22 kDa was most abundant as shown by highest intensity. Protein of 6.5 kDa remained unaffected till 60 min. There was almost no change in the amount of the proteins of mol wts 65, 22 and 20 kDa up to 15 min, whereas at 60 min decrease in intensity of all these proteins was observed. The amount of the 26 kDa protein was constant up to 8 min and thereafter it could not be observed indicating complete digestion, while the amount of protein of 70 kDa gradually decreased up to 2 min and thereafter it was completely digested.

SDS-PAGE of peanut CPE showed 11 proteins ranging from 6.5 to 100 kDa (Fig. 2.7 a, lane 2). Proteins of 66, 22, 18, 17 and 6.5 kDa were stable to SGF digestion up to 60 min (Fig. 2.7, lane 11). Proteins of 70 and 78 kDa remained undigested up to 4 min and 30 sec, respectively (Fig 2.7, lane 8 and 5) following digestion. Proteins of 100, 97, 35 and 15 kDa got digested in less than 15 sec in SGF. Protein of 66 kDa had highest intensity followed by 6.5 kDa protein as shown by densitometric analysis. The densitometry of undigested bands of peanut proteins showed that concentration of 66, 22, 18,
Fig. 2.6. (a) SDS-PAGE of the SGF digested soybean CPE.
Lane 1: Pepsin; Lane 2: soybean CPE; Lane 3: 0 min; Lane 4-11: SGF digestion pattern of soybean at time points: 0.25, 0.5, 1, 2, 4, 8, 15 and 60 min, Lane M: Molecular weight markers.
(b) Quantification of density of undigested soybean protein bands.
Values are means of triplicate analysis.
Fig. 2.7. (a) SDS-PAGE of the SGF digested peanut CPE. Lane 1: Pepsin; Lane 2: peanut CPE; Lane 3: 0 min; Lane 4-11: SGF digestion pattern of peanut at time points: 0.25, 0.5, 1, 2, 4, 8, 15 and 60 min, Lane M: Molecular weight markers.
(b) Quantification of density of the undigested peanut protein bands. Values are means of triplicate analysis.
17 and 6.5 kDa remained almost constant till 60 min while protein bands of 70 and 78 kDa showed continuous decrease with increase in incubation period and degraded completely after 4 min and 30 sec, respectively (Fig. 2.7 b).

SDS-PAGE of chickpea CPE showed 10 proteins with mol wts ranging from 18 to 84 kDa (Fig. 2.8 a, lane 2). Five proteins of 55, 45, 35, 20 and 18 kDa remained undigested till 60 min in SGF (Fig. 2.8 a, lane 11). Proteins of 70 kDa and 64 kDa degraded completely after 15 min (lane 10). A new protein band of approximately 42 kDa appeared after 15 sec of digestion (Fig. 2.8 a, lane 4) and was stable up to 60 min (Fig. 2.8 a, lane 11). This 42 kDa protein was not present in the CPE of chickpea and may be the hydrolyzed product of some larger protein. Proteins of 84, 38 and 29 kDa were easily digested in SGF in less than 15 sec. Fig. 2.8 b shows the time course of digestion proteins determined by densitometry of 70, 64, 55, 45, 42, 35, 20 and 18 kDa proteins of chickpea. The protein of 35 kDa was most abundant and amount of 35 and 20 kDa proteins remained almost constant up to 60 min while that of 70, 64, 55 and 18 kDa constantly decreased with time. Intensity of 42 kDa protein increased with time up to 60 min.

The digestion profile of black gram is shown in Fig. 2.9 a. SDS-PAGE of its CPE resulted in 13 proteins with mol wts ranging from 6.5 to 84 kDa (Fig. 2.9 a, lane 2). Protein of 47 kDa was stable up to 60 min after SGF digestion (Fig. 2.9 a, lane 11). Proteins of 30, 29, 28, 26, 24, 22 and 16 kDa were stable till 15 min (Fig. 2.9 a, lane 10). Proteins of 14 and 12 kDa were digested within 2 min (Fig. 2.9 a, lane 7). Proteins of 84, 66 and 6.5 kDa were digested rapidly in less than 15 sec. Fig. 2.9 b shows densitometric analysis of remainder of undigested proteins of 47, 30, 29, 28, 26, 24, 22, 16, 14 and 12 kDa of black gram. The protein of 47 kDa was most abundant, followed by 30 kDa and 16
Fig. 2.8. (a) SDS-PAGE of the SGF digested chickpea CPE.
Lane 1: Pepsin; Lane 2: chickpea CPE; Lane 3: 0 min;
Lane 4-11: SGF digestion pattern of chickpea at time points: 0.25, 0.5, 1, 2, 4, 8, 15 and 60 min,
Lane M: Molecular weight markers.
(b) Quantification of density of the undigested chickpea protein bands.
Values are means of triplicate analysis.
Fig. 2.9. (a) SDS-PAGE of the SGF digested black gram CPE. Lane 1: Pepsin; Lane 2: black gram CPE; Lane 3: 0 min; Lane 4-11: SGF digestion pattern of black gram at time points: 0.25, 0.5, 1, 2, 4, 8, 15 and 60 min, Lane M: Molecular weight markers.
(b) Quantification of density of the undigested black gram protein bands. Values are means of triplicate analysis.
The amount of 47 kDa protein decreased suddenly after 15 sec and was almost constant up to 60 min. There was a constant decrease in the amount of 30, 29, 28, 26, 24, 22 and 16 kDa proteins with time and they were fully digested by 15 min. The amounts of 14 and 12 kDa proteins decreased with time up to 2 min, after that both were completely digested as these could not be seen after staining.

Fig. 2.10 a, shows digestion profile of kidney bean. SDS-PAGE of kidney bean CPE showed 8 proteins with mol wt ranging from 6.5-116 kDa (Fig. 2.10 a, lane 2). Protein bands of 45, 29, 24, 20 and 6.5 kDa were stable up to 1 h after SGF digestion (Fig. 2.10 a, lane 11). Remaining of the three proteins of 116, 84 and 27 kDa were digested in less than 15 seconds. Protein of 45 kDa was most abundant, followed by 29 kDa. There was decrease in the amount of all the five pepsin stable proteins of 45, 29, 24, 20 and 6.5 kDa at 60 min in comparison to the earlier time points (Fig. 2.10 b).

SDS-PAGE of Bengal gram CPE showed eight proteins with mol wt ranging from 20 to 97 kDa (Fig. 2.11 a, lane 2). Out of eight, only one protein of approximately 20 kDa remained stable till 2 min, as shown by SGF digestion and densitometric analysis (Fig. 2.11 a, lane 7 and Fig. 2.11 b). Remaining seven proteins were digested in less than 15 seconds.

SDS-PAGE of red gram CPE resulted in nine proteins of mol wt 18 to 130 kDa (Fig. 2.12 a, lane 2). Out of nine, two proteins of 45 and 30 kDa were stable up to 60 mins, whereas 66 kDa proteins got digested after 2 mins, as shown by SGF digestion (Fig. 2.12 a, lane 11 and 7). Fig. 2.12 b shows densitometric analysis of pepsin stable 66, 45 and 30 kDa proteins. Amount of 45 kDa protein was abundant, followed by 66 kDa. Protein of 66 kDa digested completely after 2 min as revealed by densitometric analysis. Intensity of 45 kDa protein was almost unchanged up to 15 min; however
Fig. 2.10. (a) SDS-PAGE of the SGF digested kidney bean CPE. Lane 1: Pepsin; Lane 2: kidney bean CPE; Lane 3: 0 min; Lane 4-11: SGF digestion pattern of kidney bean at time points: 0.25, 0.5, 1, 2, 4, 8, 15 and 60 min, Lane M: Molecular weight markers. (b) Quantification of density of the undigested kidney bean protein bands. Values are means of triplicate analysis.
Fig. 2.11. (a) SDS-PAGE of the SGF digested Bengal gram CPE. Lane 1: Pepsin; Lane 2: Bengal gram CPE; Lane 3: 0 min; Lane 4-11: SGF digestion pattern of Bengal gram at time points: 0.25, 0.5, 1, 2, 4, 8, 15 and 60 min, Lane M: Molecular weight markers.

(b) Quantification of density of the undigested Bengal gram protein bands. Values are means of triplicate analysis.
Fig. 2.12. (a) SDS-PAGE of the SGF digested red gram CPE.
Lane 1: Pepsin; Lane 2: red gram CPE; Lane 3: 0 min; Lane 4-11: SGF digestion pattern of red gram at time points: 0.25, 0.5, 1, 2, 4, 8, 15 and 60 min, Lane M: Molecular weight markers.
(b) Quantification of density of the undigested red gram protein bands. Values are means of triplicate analysis.
after 15 min its intensity was drastically reduced. Amount of 30 kDa protein remain almost unchanged up to 60 min in SGF.

SDS-PAGE of green gram CPE resulted in eighteen proteins from 18 to 170 kDa (Fig. 2.13 a, lane 2). Out of eighteen, three proteins of 52, 35 and 30 kDa were stable up to 60 mins (Fig. 2.13 a, lane 11) whereas 18 and 38 kDa proteins got digested after 2 and 15 mins, respectively (Fig. 2.13 a, lane 7 and 10) following SGF digestion. A new protein band of 20 kDa appeared after 15 sec (lane 5) and it was found to be stable up to 60 min in SGF (Fig. 2.13 a, lane 11). Fig. 2.13 b shows densitometric analysis of pepsin resistant proteins of green gram. Protein of 52 kDa was abundant and it was found to be stable up to 60 min, however its intensity was continuously decreased through-out the time. Amount of 35, 30 and 20 kDa proteins was almost constant up to 60 mins, whereas 38 and 18 kDa proteins were completely digested after 15 and 2 min, respectively as these could not be seen after staining.

2.4. Discussion
Stability to digestion against gastric enzymes is thought to be an important feature of allergenic food protein (Burks et al., 1992; Astwood et al., 1996; Vieths et al, 1999; Besler et al., 2001). Stable proteins may be important for both sensitization of the immune system after reaching intestinal mucosa and for the elicitation of gastrointestinal and other symptoms of food allergy (Vieths et al., 1999). This assumption was validated in this study using known allergenic and non-allergic purified food proteins. Our results with purified allergenic proteins like lysozyme, ovalbumin and β-lactoglobulin showed stability in SGF for more than 8 minutes while non-allergic proteins like β-amylase and lipoxigenase were digested within 15 seconds,
Fig. 2.13. (a) SDS-PAGE of the SGF digested green gram CPE.
Lane 1: Pepsin; Lane 2: green gram CPE; Lane 3: 0 min;
Lane 4-11: SGF digestion pattern of green gram at time points: 0.25, 0.5, 1, 2, 4, 8, 15 and 60 min,
Lane M: Molecular weight markers.
(b) Quantification of density of the undigested green gram protein bands.
Values are means of triplicate analysis.
which are in agreement with the earlier studies of Astwood et al. (1996) and Thomas et al. (2004).

A number of allergens from soybean and peanut has been identified and characterized (Helm et al., 2000; Koppelman et al., 2003). Therefore, soybean and peanut were taken as model leguminous crops to see the usefulness of pepsin stability in screening the allergenic proteins in legume CPEs, *in vitro* digestibility of CPE of these two food crops; soybean and peanut was performed. Chickpea and black gram CPE whose allergens are not well characterized were investigated for digestibility. The SGF digestibility of kidney bean, bengal gram, red gram and green gram CPE was performed to identify non-digestible proteins, which may probably be allergenic.

Among all five proteins of soybean that were stable to pepsin, four proteins of 65, 22, 20 and 6.5 kDa of soybean CPE remained undigested in SGF upto 60 min. On the basis of mol wts, these proteins probably correspond to soybean major allergen alpha subunit of Gly mBd 60 K allergen (Ogawa et al., 2000), G2 glycinin (Helm et al., 2000), soybean minor allergen; Kunitz-trypsin inhibitor (Roychaudhuri et al., 2004) and Gly m2 allergens (Codina et al., 1997), respectively. The 26 kDa protein was stable upto 8 min and may possibly be Gly m Bd 28 K (Tsuji et al., 1997).

Like soybean, mol wts of peanut proteins stable in SGF were matched. There was a correlation between stability and allergenicity as peanut CPE electrophoresis post-SGF digestion resulted in 66, 18, 17 and 7 kDa bands (stable upto 60 min) that have similar mol wt to major allergen Ara h1 (Viquez et al., 2003), oleosin monomer (Pons et al., 2002), Ara h2 (Burks et al., 1992), tyrosin and chymotrypsin inhibitor (Clarke et al., 1998), respectively.
Chickpea CPE SGF digestion resulted in seven non-digestible proteins of 70, 64, 55, 45, 35, 20 and 18 kDa. Proteins of similar mol wts have been shown to be allergenic by other investigators using immunoblot (Niphadkar et al., 1997; Patil et al., 2001) and thus substantiate the hypothesis that non-digestible proteins may be allergenic. In black gram protein of 47 kDa which was stable up to 60 min and proteins of 29, 28, 26, 24, 22, 16, 14 and 12 kDa were stable from 2 to 15 min in SGF may be allergenic as most of them have similar mol wt to IgE binding proteins of black gram reported by Kumari et al (2006). In Bengal gram, 20 kDa and in kidney bean, 50, 29, 24, 20 and 6.5 kDa proteins were found to be stable after SGF digestion. In red gram CPE digestion, 45 and 30 kDa proteins persisted up to 60 min in SGF while in green gram, 52, 38, 35, 30 and 18 kDa proteins were stable in SGF. All these proteins need to be tested for the allergenic potential as stability in SGF has been correlated with allergenicity (Astwood et al., 1996).

In this study, we are reporting for the first time non-digestible proteins of different legume crops as evident by protein profile after SGF digestion. It was found that seven proteins from chickpea (70, 64, 55, 45, 35, 20 and 18 kDa), ten from black gram (47, 30, 29, 28, 26, 24, 22, 16, 14 and 12 kDa), five from kidney bean (45, 29, 24, 20 and 6.5 kDa), one protein of 20 kDa from bengal gram, three from red gram (66, 45 and 30 kDa) and five from green gram (52, 38, 35, 30 and 18 kDa) were stable after SGF digestion. All these proteins from different leguminous crops may have allergenic potential and needs to be tested for their allergenicity using sera of patients having allergy to these crops. Correlation between mol wts of undigested proteins of soybean, peanut, chickpea and black gram coincided to their reported allergens. These results depict utility of simple SGF digestion as fast screening method for evaluating allergenicity of a legume protein. Further
studies are required to characterize these non-digestible proteins of different leguminous crops, so that it may find application in the prevention and treatment of allergic reactions in sensitized individuals. This study may also help in the development of hypoallergic genetically modified legumes.

2.5. Conclusion
All the selected legumes namely; chickpea, green gram, red gram, black gram, kidney bean and bengal gram have one or more proteins stable to in vitro pepsin digestion. Non-digestible proteins need further evaluation to test their allergenic potential. Identification of non-digestible protein may be useful in predication of allergenic response.