

COMPOSITION ANALYSIS OF SOME SELECTED LEGUMES FOR PROTEIN ISOLATES RECOVERY

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ABSTRACT

Four different legumes *i.e.* chickpea, lentil, broad and kidney beans were evaluated for chemical components, antinutritional profiling and protein isolates recovery including yield. In tested legumes, antinutritional compounds like phytates and trypsin inhibitor activities were higher in kidney bean whereas, haemagglutinin-lectin content in broad bean. However, trypsin inhibitor activity was lowest in lentil after hydration followed by cooking treatment. Proximate composition revealed that lentil contained crude protein content $31.12 \pm 1.68\%$, followed by chickpea ($22.83 \pm 1.07\%$), broad bean ($22.61 \pm 1.24\%$) and kidney beans ($20.09 \pm 0.52\%$). The isoelectric precipitation method was used for the recovery of respective protein isolates from defatted legume samples. Lentil and chickpea showed higher protein isolates recovery, and yield as compared to other legumes. Electrophoretic identification explicated that legume protein isolates polypeptide bands fall within the range of 4-70kDa. It is deduced that lentil and chickpea had better protein isolates recovery and yield thus suitable for the preparation of protein enriched food formulations.

Keywords: Legumes, Protein, Antinutritional factors, Protein isolates, SDS-PAGE.

INTRODUCTION

Legumes play an important role in human nutrition as they are rich source of protein, calories, certain minerals and vitamins (Baloch and Zubair, 2010). These are crops of the family *Leguminosae* that is also called *Fabaceae*. Grain legumes are used as pulse (*dhal*) in Indo-Pak region (Khan *et al.*, 2009). It is well documented that cereal proteins are deficient in certain essential amino acids, particularly in lysine (Anjum *et al.*, 2005) whereas legumes contained adequate amount of lysine (Sai-Ut *et al.*, 2009). It is advisable to enhance the protein content of the diet through easily available and accessible plant protein sources especially legumes to improve the nutritional status of the low-income groups of population (Khattab and Arntfield, 2009).

Pakistan being an agro-based economy produces different types of legumes like chickpea, lentil, broad and kidney beans etc. Utilization of legumes in food formulations as a source of protein is increasing as they provide balanced amino acids profile. The nutritional quality depends upon specific amino acids and their physiological utilization after digestion, absorption and minimal mandatory rates of oxidation (Longnecker *et al.*, 2002). Thus the protein energy malnutrition threats in developing nations can be minimized using protein enriched sources in daily diet.

The antinutrients like trypsin inhibitors, phytic acid, saponins, haemagglutinins and tannins are some of the undesirable components in legumes that could hinder utilization of important minerals including

calcium, magnesium, iron and zinc etc. It interferes with their absorption & utilization and thereby contributes to mineral deficiency (Vasagam and Rajkumar, 2011). Protease inhibitors in various legumes have ability to retard proteolytic enzyme activity. Lectins are polymeric proteins present in common beans that bind to monosaccharide in glycoproteins of the cell membrane, causing lesions in the intestinal mucosa and reduced nutrient absorption (Ma *et al.*, 2011). Moreover, dehulling, soaking, germination, cooking and roasting have been shown to exert beneficial effects on nutritional quality of legumes. Previously, different processing methods such as boiling, hydration and germination are used to inactivate the antinutrients (Shimelis and Rakhshit, 2007) in the plant based foods thus enhances the nutritional value of isolated protein (Agbede *et al.*, 2005).

Earlier, Rangel *et al.*, (2004) indicated that cowpea protein isolates (CPI) were obtained by isoelectric precipitation from defatted cowpea meal. Proteins content of isolated protein from chickpea flour by isoelectric precipitation technique ranged from 84.8-87.8% (Paredes-Lopez *et al.*, 2006). Additionally, protein isolates with meek flavor are used in wide range of applications including complimentary foods, remedial ingredient for cancer cure and cardiovascular diseases (Davis, 2004). Heat treated white bean and chickpea are used for protein extraction (Arcan and Yemencioğlu, 2007).

In present study, some legumes were evaluated for proximate composition and antinutritional compounds. Accordingly, protein contents of the selected

legumes were also assessed to analyze their recovery and yield. This information will be helpful to the food technologists for developing protein enriched formulation for community.

MATERIALS AND METHODS

Materials: In the present study, legume seeds (chickpea, lentil, broad and kidney beans) were collected from Pulses Research Institute, Ayub Agricultural Research Institute, Faisalabad. Three homogenous replicates of each legume were made for further analysis. For statistical analysis, completely randomized design (CRD) at 5% level of significance was used. Reagents were procured from Merck (Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan). Legumes were cleaned to remove dust and other foreign materials, manually.

Antinutrients: The triplicate samples of each selected legumes were homogeneously mixed with calcium hydroxide solution (20%w/w) at room temperature for analysing antinutrients contents. Haemagglutinin lectin activity was estimated by Rabbit Erythrocyte Agglutination Test (Benedito-de and Barber, 1978). Lectin activity was determined in haemagglutinin units (HU) as indicated by Tan *et al.* (1983). For trypsin inhibitor, samples were mixed with 0.05N HCl in a Sorvall Omni Mixer (Ivan Sorvall, Inc., Newtown). The resultant slurry was centrifuged (M-3k30, Sigma, Germany) at 4000 rpm followed by addition of trichloroacetic acid (TCA) to the supernatant and recentrifuged. After neutralization, the enzyme inhibitory response was assessed (Decker, 1977). Phytic acid content of legume samples was determined by following the protocol of Haug and Lantszch (1983). Prepared samples were heated with acidic ammonium iron-III sulphate solution of known concentration. The reduction in iron content of supernatant was indicated as phytate content using 2,2 bipyridine at wavelength 519nm through spectrophotometer (CE 7200-7000 series, Cecil, UK).

Inactivation of Antinutrients in Legumes: Inactivation of antinutrients in the selected legumes was carried out following the method of Shimelis and Rakshit (2007). Legume samples (100g) were soaked at room temperature for 12 h (overnight) in distilled water (pH 6.9) and sodium bicarbonate solution (0.05%, pH 8.2). The seed-to-solution ratio was 1:3 (w/v). The unabsorbed liquid was drained off, treated seeds were dipped twice in distilled water. After soaking for 12 h, the seeds were rinsed with distilled water and cooked (water = two times the weight of soaked seeds) followed by cooking of pre-soaked seeds was performed at 97 °C. The cooking water was drained off, and the seeds were rinsed twice in distilled water, crushed and lyophilized at temperature -

60°C under vacuum 0.03 bar (Freeze dry system, Christ, Germany). The lyophilized samples were ground (60mesh) and stored in air tight bottles at 4°C for further analysis.

Proximate Analysis: Legume samples were analyzed for crude protein (Method no. 46-30), crude fat (Method no. 30-25), crude fiber (Method no. 32-10), ash (Method no. 08-01) and nitrogen free extract (NFE) on dry weight basis following their respective protocols mentioned in AACC (2000).

Preparation of Legume Protein Isolates: Protein isolates were prepared following the method of Makri *et al.* (2005) as mentioned in Figure 1. The flour of different legumes was defatted with n-hexane followed by dispersion in distilled water (1/10) and pH adjusted at 9.5. After centrifugation (4000rpm) for 20min, supernatant will be collected. Later, the supernatant was adjusted to pH 4.5 for protein precipitation recovered by recentrifugation then neutralized and freeze dried.

Protein Isolates Assay

Isolate Recovery: Recovery of legume protein isolates were determined as weight of protein isolates attained after isoelectric precipitation per 100g weight of respective legume (Wang *et al.*, 1999).

Protein Content: The nitrogen percentage was measured by Kjeltach System and the crude protein was calculated by multiplying percent nitrogen with conversion factor 6.25.

Protein Yield: For the determination of protein yield following expression was used (Wang *et al.*, 1999).

$$\text{Yield} = \frac{\text{Isolate}}{\text{recovery}} \times \frac{\text{Isolate protein (\%)}}{\text{Legume protein (\%)}} \times 100$$

Gel Electrophoresis (SDS-PAGE): Prepared legume protein isolates were solubilized in sample buffer (250µL). The electrophoresis was performed using 12.5% separating and 4% stacking gel on Bio-Rad Mini Protean 3 System (Bio-Rad Laboratories, Hercules, CA, USA). The loaded gels were run at constant voltage (60V) for 2.5h till the front dye shifted thoroughly far down the gel. Staining of gels was carried out by using Coomassie Brilliant Blue (CBB) and de-stained with methanol-water mixture (Tang and Sun, 2011).

Statistical analysis: The collected data was statistically analyzed through Statistical Package (Costat-2003, Co-Hort, v 6.1.). Means were further compared through Duncan Multiple Range test (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

Antinutritional Factors:

Phytates: Means in Table 1 indicated that higher phytates content were present in kidney bean

(21 ± 0.54 mmol/kg) followed by broad bean (20.50 ± 1.12 mmol/kg), lentil (3.50 ± 0.21 mmol/kg) and chickpea (1.00 ± 0.05 mmol/kg). Heat treatment dissociated phytate complex effectively thereby reducing phytate content in legume samples. The highest reduction for this attribute was observed in kidney bean (6.54 ± 0.17 mmol/kg) followed by broad bean (4.73 ± 0.26 mmol/kg), lentil (0.73 ± 0.03 mmol/kg) and chickpea (0.31 ± 0.01 mmol/kg). Earlier, Almeida *et al.* (2008) they reported that broad bean contains phytate ranging from 12.7 to 14.3 mmol/kg and trypsin inhibitor activity from 4.12 to 4.38 TIU/mg. Similarly, it is well documented that different types of the heat treatments can significantly reduce the antinutritional compounds of the legumes (El-Adawy, 2002).

Haemagglutinin-Lectin: Heat labile haemagglutinin is growth depressant even at lower level in foods and tends to be toxic at higher concentration (Liener *et al.*, 1994). It is obvious from the statistical results that haemagglutinin-lectin content was affected significantly with heating. The highest haemagglutinin-lectin content were observed in broad bean (48.50 ± 2.65 activity/mg) followed by lentil (20.45 ± 1.83 activity/mg), chickpea (5.25 ± 0.25 activity/mg) and kidney bean (1.90 ± 0.05 activity/mg) as shown in Table 1.

It has been observed from the current study that heat treatment significantly decreased haemagglutinin-lectin. The highest decrease for this parameter occurred in broad bean (10.90 ± 0.50 activity/mg) followed by chickpea (1.09 ± 0.05 activity/mg), lentil (0.89 ± 0.05 activity/mg) and kidney bean (0.88 ± 0.02 activity/mg) as shown in Table 1. Moreover, there was a marked reduction in lectin activation owing to raw material heating. Germination and soaking reduce haemagglutinin-lectin in broad bean (Alonso *et al.*, 2000). Microwave heat treatment significantly reduces haemagglutinin-lectin activity in beans and legumes (El-Adawy, 2002).

Trypsin Inhibitor: Means showed that maximum trypsin inhibitor activity was in chickpea (10.50 ± 0.49 TIU/mg) followed by lentil (7.50 ± 0.37 TIU/mg) and kidney bean (4.01 ± 0.10 TIU/mg). However, minimum value was observed in broad bean (3.35 ± 0.18 TIU/mg) as shown in Table 1. The highest decrease in trypsin inhibitor was detected in chickpea (2.00 ± 0.09 TIU/mg) followed by kidney bean (1.95 ± 0.05 TIU/mg), broad bean (1.07 ± 0.06 TIU/mg) and lentil (0.51 ± 0.03 TIU/mg). Among antinutrients, trypsin inhibitor has received core attention and reported to cause growth depression, poor feed efficiency and inhibits digestive enzymes (Bahnansey *et al.*, 1986). However, they can be inactivated at elevated temperature (Liener *et al.*, 1994).

The current findings are in agreement with Olivera-Castillo *et al.* (2007), they recorded noticeable decrease in trypsin inhibitor in dry heated cowpea meal.

Previously, it was reported that heat treatment readily inactivates trypsin inhibitor in rice bran (Tashiro and Ikegami, 1996). Microwave, dry and moist heat treatments are capable of reducing trypsin inhibitor (Deolankar and Singh, 1979). Present research results are in agreement with El-Adawy (2002) who observed reduction in trypsin inhibitor activity depends on heating conditions provided to legumes.

Proximate Composition: Proximate assay is an important criterion to assess the overall composition and nutritional status of any ingredient intended for food use. In this context, legumes flour was analyzed for different quality attributes such as moisture, crude protein, crude fat, crude fiber, ash and nitrogen free extract (NFE). Proximate composition on dry weight bases (Table 2) showed significant variations among legume samples for moisture, crude protein, crude fat, crude fibre and ash content whereas, NFE exhibited non significant effect in the evaluated legumes. Moisture content ranged from 8.01 ± 0.38 to $12.97 \pm 0.71\%$ in different tested legumes. However, value for protein content remained high in lentil ($31.12 \pm 1.68\%$) followed by chickpea ($22.83 \pm 1.07\%$), broad bean ($22.61 \pm 1.24\%$) and kidney bean ($20.09 \pm 0.52\%$). The highest crude fiber and ash were recorded in kidney bean 6.78 ± 0.17 and $3.85 \pm 0.10\%$, respectively.

Present findings regarding proximate composition are in conformity with values described in previous literature; however, slight variations may be due to varietal differences and environmental conditions. Chemical composition of lentil was also analyzed by Suliman *et al.* (2006) and observed that moisture, protein, fat, fiber and ash were 7-10, 32-33, 1-2, 2-4 and 2-3%, correspondingly in defatted samples of respective legume varieties. In another research, relative composition of moisture, protein, fat and ash of chickpea remained as 9.00, 23.08, 6.65 and 3.21%, respectively (Aurelia *et al.*, 2009).

Current results for broad bean samples are also in resemblance with the findings of Tsoukala *et al.* (2006); found that moisture, crude protein, crude fat and ash were 14, 26.7, 1.6 and 2.8%, respectively in different legumes. The data pertaining to present study are closely associated with the work of Mortuza *et al.* (2009), they delineated 30.5, 3.22 and 3.61% crude protein, crude fat and ash contents, respectively.

In array of investigations, variations in proximate composition of different legumes as lentil, chickpea and kidney bean have been observed owing to different environments, genotype and analytical methods. It was further reported that protein content was sensitive to rainfall, light intensity, length of growing season, day duration, temperature and agronomic practices (Bampidis and Christodoulou, 2011). As far as present study is concerned, variations in the composition among the

reported legumes are attributed to utilization of indigenous varieties. Earlier, Zia-ur-Rehman and Shah (2005) worked on beans and legumes as a source of protein, fiber, starch and other nutritional components. They elucidated that legumes provide good quality protein that acts as major contributor amongst phyto-proteins.

Protein Isolates Recovery and Yield: Mean values for protein recovery and yield have been depicted in Table 3. Maximum protein isolates recovery was revealed in lentil protein isolates (LPI) followed by chickpea protein isolates (CPI) and broad bean protein isolates (BPI). Nevertheless, the lowest protein isolates recovery was in kidney bean protein isolates (KPI). Similarly, the highest crude protein was found in isolates of LPI followed by CPI, BPI and KPI. The highest protein yield (80.47±5.71%) was recorded in LPI whilst, 73.14±3.44 and 67.58±3.70% for CPI and BPI, respectively. However, the lowest yield of 52.83±3.36% was observed in KPI.

Present results regarding recovery of legume protein isolates are in harmony with the findings of Khan *et al.* (2011) showing 16.84±0.22 to 18.32±0.29g/100g recovery of protein isolates. Protein yield of KPI is smaller than LPI owing to protein-protein interactions. The results are also matched with the findings of Rodriguez-Ambriz *et al.* (2005), they found appreciable protein isolates yield in *Lupinus campestris* legume. The present results of protein isolates yield from chickpea and lentil are in accordance with the findings of Boye *et al.* (2010) as they observed 69.1% yield in chickpea. The highest crude protein was observed for lentil protein isolates.

The instant results are in agreement with the findings of Suliman *et al.* (2006), that the lowest value for KPI is in resemblance with the findings of Leon *et al.* (2007), reported 71% crude protein content. In another study, chickpeas and lentil were assessed for their protein content using alkaline extraction followed by acid precipitation. Contrarily, it was observed that chickpea flour yield higher protein content than that of lentil (Alsohaimy *et al.*, 2007).

In the nutshell, legume protein isolates with significant yield are important to be incorporated in protein based products development. Thereby, have tendency to be used as an alternate in the preparation of novel foods. Moreover, protein isolates from indigenous legumes can share the burden of protein demand among the masses.



Figure 1: Systematic flow sheet for legume protein isolates recovery

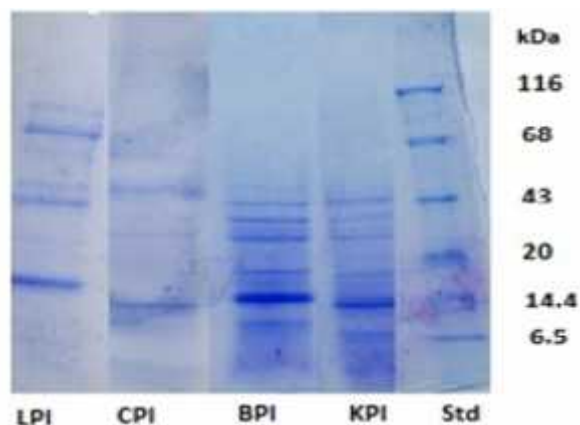


Figure 2: Electrophorogram of lentil protein isolates (LPI), chickpea protein isolates (CPI), broad bean protein isolates (BPI), kidney bean protein isolates (KPI) and standard (Std).

Table 1. Antinutritional factors of legumes

Legumes	Phytates (mmol/kg)		Haemagglutinin-lectin activity/mg		Trypsin inhibitor activity/mg	
	(Before treatment)	(After treatment)	(Before treatment)	(After treatment)	(Before treatment)	(After treatment)
Chickpea	1.00±0.05 ^c	0.31±0.01 ^d	5.25±0.25 ^c	1.09±0.05 ^b	10.50±0.49 ^a	2.00±0.09 ^a
Lentil	3.50±0.21 ^b	0.73±0.03 ^c	20.45±1.83 ^b	0.89±0.05 ^b	7.50±0.37 ^b	0.51±0.03 ^c
Broad bean	20.50±1.12 ^a	4.73±0.26 ^b	48.50±2.65 ^a	10.90±0.50 ^a	3.35±0.18 ^c	1.07±0.06 ^b
Kidney bean	21.00±0.54 ^a	6.54±0.17 ^a	1.90±0.05 ^c	0.88±0.02 ^b	4.01±0.10 ^c	1.95±0.05 ^a

Means sharing the same letter in a column are not significantly different ($p > 0.05$)

Table 2. Proximate composition (%) of legumes

Legumes	Moisture	Crude protein	Crude fat	Crude fiber	Ash	NFE
Chickpea	8.01±0.38 ^b	22.83±1.07 ^b	5.43±0.26 ^a	3.50±0.16 ^b	3.04±0.14 ^b	57.19±2.69
Lentil	9.14±0.78 ^b	31.12±1.68 ^a	0.81±0.04 ^c	3.68±0.43 ^b	2.62±0.31 ^c	52.63±2.22
Broad bean	12.97±0.71 ^a	22.61±1.24 ^b	2.67±0.15 ^b	2.46±0.13 ^c	2.90±0.16 ^{bc}	56.39±3.08
Kidney bean	9.15±0.24 ^b	20.09±0.52 ^b	2.46±0.06 ^b	6.78±0.17 ^a	3.85±0.10 ^a	57.67±1.48

Means sharing the same letter in a column are not significantly different ($p>0.05$)

Table 3. Protein isolates recovery and yield

Legumes protein Isolates	Protein isolates recovery (g/100g legume)	Crude Protein (%)	Protein yield (% legume protein)
Chickpea protein isolates	20.70±0.97 ^b	80.67±3.80 ^{ab}	73.14±3.44 ^{ab}
Lentil protein isolates	29.58±1.49 ^a	84.66±6.48 ^a	80.47±5.71 ^a
Broad bean protein isolates	19.68±1.08 ^b	77.64±4.25 ^{ab}	67.58±3.70 ^b
Kidney bean protein isolates	14.60±0.38 ^c	72.69±3.87 ^b	52.83±3.36 ^c

Means sharing the same letter in a column are not significantly different ($p>0.05$)

SDS-Page: The electrophorogram of SDS-PAGE for legumes including chickpea, lentil, broad and kidney bean protein isolates along with standard has been presented in Figure 2. The respective documentation showed that legume protein isolates bands were in the range of 4 to 70kDa and followed the similar pattern as reported earlier. Several low molecular weight fractions were also evident in the electrophorogram. For LPI, proteins are comprised of several polypeptide bands fall between 18.4 to 70kDa. In case of CPI, the bands lie between 10.6 to 44.6kDa. For BPI the bands ranged from 8 to 44.8kDa while for KPI the protein bands were between 4 to 45kDa.

Present results are associated with the findings of Kimura *et al.* (2008), they found similar protein profile in other legumes like pea, fava bean, cowpea and french bean. Likewise, Tavano and Neves (2008) reported that chickpea native globulin with a molecular weight 140kDa was resolved in sodium dodecyl sulfate polyacrylamide gel electrophoresis in seven polypeptide bands in the range of 12.4 and 67kDa. Earlier, proteins were isolated from chickpea flour by micellization and isoelectric precipitation techniques. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed a molecular weight distribution between 16.6 and 66.4kDa for micelle and 14.9 and 84.2kDa for isoelectric proteins (Paredes-Lopez *et al.*, 2006).

Conclusively, from nutritional point of view, tested legumes as lentil, chickpea, kidney & broad bean are good alternative sources of protein. The activity of antinutritional compounds present in legumes was significantly reduced through hydration followed by cooking. Protein isolates recovery, isolates crude protein content and protein yield were higher in lentil followed by chickpea, broad bean whilst the lowest estimation was observed in kidney bean. Moreover, these legumes polypeptide band are in the range of 4 to 70kDa. It is

deduced that lentil and chickpea protein isolates have better potential to be used in protein enriched food formulations.

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