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Nutritional and Functional Properties of Some Promising Legumes Protein Isolates

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Abstract: Proteins are essential component of diet performing multifarious role in human body. Present project was an attempt to extract and characterize legumes protein isolates for their functional properties. Four different legumes i.e. cowpea, pigeon pea, peas and mungbean were evaluated for protein content, functional properties and their ability to improve nutritional quality of foods. Cowpea exhibited maximum protein content 27.88±1.95% followed by mungbean, peas and pigeon pea. As for as functional properties are concerned, cowpea protein isolates showed highest bulk density 0.71±0.05 g/cm^3 however, maximum protein solubility 82±4.97 was observed in pea protein isolates. Maximum water and oil absorption capacity 163±10.05, 168±11.72% was observed in mungbean and pigeon pea protein isolates, respectively. Likewise different legumes protein isolates showed significant results for emulsifying, foaming and gelling properties.

Key words: Nutritional profile, functional properties, protein isolates, legumes

INTRODUCTION

Protein malnutrition is one of the major nutritional problems in the developing world. The specific maladies like kwashiorkor and marasmus are prevalent in the children owing to protein deficiency, whereas in adults, results in poor health and reduced work capacity. Bridging the gap between increased food consumption and production is amongst the most challenging tasks round the globe especially in developing countries (Black *et al*., 2008). The existing problems of food security and malnutrition coupled with escalating population, uncertain crop yield and high cost of animal based food supplies have urged to identify and incorporate unconventional protein sources to enrich the traditional formulations (Awan, 2000). Generally there are two main sources of protein i.e. animal and plant; provision of adequate animal proteins is difficult due to high cost and changing consumer's attitudes towards animal based proteins. Consumers are more conscious in their food selection owing to growing awareness about nutritional dependent ailments. An alternative for improving protein intake of the people is to supplement the diet with plant proteins. For that reason, consumption of plant protein isolates with special reference to legumes is beneficial (Nunes *et al*., 2006; Iqbal *et al*., 2005). Legumes are inexpensive source of proteins with high nutritional profile and after cereals, important food source for humans (Vietmeyer, 1986; Doyle, 1994). Protein content in legume ranged from 17-40%, contrasting with that of cereals 7-13% and comparable to meat 18-25% (Genovese and Lajolo, 2001). Being a cheap source of protein for low income group of population, legumes are commonly used as a substitute for meat and play a significant role in alleviating the protein-energy malnutrition. In addition they are also a good source of complex carbohydrates, dietary fiber and contain significant amounts of vitamins and minerals (Morrow, 1991; Nielsen, 1991; Tharanathan and Mahadevamma, 2003). Protein isolates obtained from the legumes through isoelectrict precipitation have high percentage of protein contents, which make them potential protein sources for food industry applications and this potential usefulness will depend on their functional properties. Functional properties are the physical and chemical characteristics of the specific protein influencing its behavior in food system during processing, storage, cooking and consumption. The examples of functional properties include bulk density, protein solubility, water and oil absorption capacity, emulsifying and foaming properties. The factors that effect functional behavior of proteins in foods are their size, shape, amino acid composition and sequence, net charge, hydrophobicity, structure, molecular rigidity in response to external environment (pH, temperature, salt concentration) or interaction with other food constituents (Aluko and Yada, 1997). Proteins from legumes have gained immense importance in modern food design due to their nutritional value and favorable functional properties.

MATERIALS AND METHODS

Procurement of raw material: Cowpea, peas, pigeon pea and mungbean were purchased from the local market, while chemicals were obtained from Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan). The legumes were cleaned, washed and dried to remove extraneous

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materials. The particle size of legumes was reduced to form fine flour through Cyclotec Mill.

Proximate analysis: The flour from each legume was analyzed for moisture, ash, crude protein, crude fat, crude fiber and NFE content according to their respective methods as described in AACC (2000).

Preparation of protein isolates: Legumes Flours were defatted by slurring the sample in an organic solvent (hexane) using soxhlet apparatus. After extraction, solvent was recovered through rotary evaporator. Protein isolates from the legumes flour were prepared by the method as described by Makri *et al.* (2005). The defatted flour was dispersed in distilled water (1/10); pH was adjusted to 9.5 with the aid of 1 N NaOH and shaked for 40 min at room temperature using a mechanical shaker. Following centrifugation at 4000 rpm for 20 min supernatant was collected. The residue was collected and dispersed in distilled water (1/5) and stirred. Following centrifugation at 4000 rpm for 20 min, the respective supernatant was collected and combined with the supernatant collected from the first centrifugation and the pH was adjusted to 4.5, the precipitated protein was recovered by centrifugation at 4000 rpm for 20 min, neutralized and freeze dried.

Functional properties

Bulk density: The bulk density of protein isolates of different legumes was determined as outlined by Okaka and Potter (1977). Ten grams of protein isolates were put into 100 ml graduated cylinder and was tapped several times on the laboratory bench till the isolate stopped settling and values were expressed as g/cm^3.

Protein solubility: The protein isolates (250 mg) were homogenized in 20 ml of 0.1 M NaCl at pH 7 for 1 h followed by centrifugation at 10,000 x g for 30 min. Nitrogen contents were determined in the soluble fractions and solubility was expressed as the percentage of total nitrogen of the original sample to that of soluble fraction (Morr *et al.*, 1985).

Water absorption: The sample (3 g) was mixed with distilled water (25 ml) and placed in pre-weighed centrifuge tubes. The tubes were stirred and centrifuged for 25 min at 3000 x g after 30 min interval. The supernatant was removed by 25 min drainage at 50°C, and protein isolate sample was re-weighed. Water absorption capacity was expressed as the number of grams of water absorbed per gram of sample (Sosulski *et al.*, 1976).

Oil absorption: The sample (0.5 g) was mixed with corn oil (6 ml) in preweighed centrifuge tubes. The tubes were stirred for one minute to get the complete dispersion of the sample in the oil. After 30 min holding time, the sample was centrifuged at 3000 x g for 25 min. 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 oil absorption capacity was expressed as grams of oil absorbed per gram of the sample (Sosulski *et al.*, 1976).

Emulsifying activity and stability: Protein isolate (3.5 g) was homogenized for 30 sec in 50 ml water using homogenizer at approximately 10,000 rpm. Corn oil (25 ml) was added and the mixture was homogenized again for 30 sec. The emulsion was divided into two equal volume aliquots and centrifuged at 1100 x g for 5 min; one aliquot was heated for 15 min at 85°C according to the procedure of Naczk *et al.* (1985). The ratio of the height of emulsion layer to the height of liquid layer was noted to calculate emulsion activity. The emulsion stability was expressed as the percentage of emulsifying activity remaining after heating (Naczk *et al.*, 1985).

Foaming capacity and stability: The capacity and stability of foams were determined by the method of Lin *et al.* (1974). A 50 ml of 3% (w/v) dispersions of protein isolate sample in distilled water were prepared and immediately transferred into graduated cylinder; volume was recorded before and after whipping. Foaming capacity was expressed as the percentage volume induced by whipping. The change in volume of foam after 60 min of standing at room temperature was recorded as foam stability.

Least gelation concentration (LGC): The LGC was determined by heating suspensions of protein isolates 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20% (w/v) for 1 h in boiling water followed by swift cooling under cold running water. The tubes were further cooled at 4°C for 2 h. LGC is the concentration at which the sample did not slide along the test tube walls in inverted position (Sathe *et al.*, 1982).

Statistical analysis: Completely Randomized Design (CRD) was applied and results were analyzed through Analysis of Variance Technique (ANOVA) using Cohort version 6.1 (Costat-2003) to determine the level of significance (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

The present project was designed to explore the nutritional and functional quality of legumes protein. The protein isolates were prepared by isoelectric precipitation and analyzed for functional properties. The investigated parameters and their respective results are discussed below.

Proximate composition: Proximate composition is important in determining the quality of raw materials and often the basis for establishing the nutritional value and

overall acceptance of the consumers. The values for moisture contents in cowpea (10.39±0.73%) and pigeon pea (11.07±0.50%) were significantly different from those for peas (9.05±0.55) and mungbean (8.30±0.51). Cowpea exhibited maximum protein content 27.88±1.95% that was at par with mungbean (25.90±1.60%) and both of these were significantly different from pigeon pea and peas. Fat contents ranged in different legume flour samples from 1.24±0.08% to 2.03±0.09%, and highest amount was noted in pigeon pea. Cowpea contained highest amount of crude fiber (9.58±0.67) and lowest was found in mungbean (4.61±0.28%). Results for ash content demonstrated significantly higher amount (4.36±0.27%) in mungbean followed by pigeon pea (3.96±0.18) and cowpea (3.84±0.27%), respectively. However, lowest was observed in peas (3.48±0.21%). The mean values for nitrogen free extract of peas, mungbean, pigeon pea and cowpea were 65.33±3.96, 63.89±3.94, 63.80±2.90 and 57.42±4.01%, respectively (Table 1). Findings of present study are corroborated with the research investigation by other scientists. Iqbal *et al.* (2006) reported that cowpea and green peas have moisture content of 9.4 and 7.8%, protein content 24.70 and 24.90%, fat contents 2.8 and 1.5% and ash contents 4.2 and 3.6%, respectively. Whilst these parameters for cowpea and peas were lowered than that reported by Mwasaru *et al.* (1999) and Costa *et al.* (2006). Even though fiber contents were comparable to that determined by Kabas *et al.* (2007); but difference observed in the NFE contents compared with those of Akubor (2003). In case of mungbean, Mubarak (2005) reported that values obtained for moisture, protein, fat, fiber, ash and NFE were 9.75, 26.37-27.50, 1.85, 4.63, 3.76 and 59.8-62.3%, respectively. Similar findings were observed by other scientists but with slight variations (Amarteifio and Moholo, 1998; Anwar *et al.*, 2007). In this regard, Oshodi *et al.* (1993) explicated that the pigeon pea has moisture, protein, fat, ash and NFE contents 5.2, 22.40, 2.6, 5.8-3.9% and 51.70%, respectively. Results are also in agreement with the findings of Eno-Obong and Carnovale (1992) and Amarteifio *et al.* (2002). Likewise, crude fiber contents ranged from 8.2-13.0% in pigeon pea (Amarteifio *et al.*, 2002).

Protein content of protein isolates: The proteins are polymer of amino acids and their relative proportion represents its quality that is dependent on the genetic makeup of legumes. The variations in protein contents are attributed to genetic makeup of legumes along with some environmental factors. Protein contents were significantly higher in cowpea (89.25±1.28%) followed by mungbean (85.46±1.52%) and pea protein isolates (83.61±1.49%). However, least protein contents were recorded in pigeon pea protein isolates i.e. 82.92±1.28% (Table 2). The variations in protein contents of different protein isolates could possibly be due to extent of soluble proteins present in raw materials. Previously, Shand *et al.* (2007); reported that pea protein isolates have 80.70% protein. Makri *et al.* (2005) determined 79.2% of protein contents in extracted isolates. Some scientists like Mwasaru *et al.* (2000) reported higher protein content in cowpea protein isolates i.e. 91.30%, however, their results supported our findings for other legumes too as they indicated that pigeon pea protein isolates contain 82.4%, while mungbean isolates have 81% of protein (Rahma *et al.*, 2000).

Table 1: Proximate composition of legumes

Legumes	Moisture (%)	Protein (%)	Fat (%)
Pigeon pea	11.07±0.50^{a}	22.01±1.00^{b}	2.03±0.09^{a}
Cowpea	10.39±0.73^{a}	27.88±1.95^{a}	1.27±0.09^{c}
Mungbean	8.30±0.51^{b}	25.90±1.60^{a}	1.24±0.08^{c}
Peas	9.05±0.55^{b}	22.95±1.39^{b}	1.41±0.09^{b}
Legumes	Ash (%)	Fiber (%)	NFE (%)
Pigeon pea	3.96±0.18^{b}	8.19±0.37^{b}	63.80±2.90^{a}
Cowpea	13.84±0.27^{bc}	9.58±0.67^{a}	57.42±4.0^{b}
Mungbean	4.36±0.27^{a}	4.61±0.28^{d}	63.89±3.94^{a}
Peas	3.48±0.2^{c}	6.83±0.41^{c}	65.33±3.96^{a}

Table 2: Means for Protein Content (PC)

Protein Isolates	PC (%)
PPPI	82.95±1.28^{c}
CPI	89.25±1.39^{a}
MBPI	85.46±1.52^{b}
PPI	83.61±1.49^{bc}

PPPI = Pigeon Pea Protein Isolates; CPI = Cowpea Protein Isolates; MBPI = Mungbean Protein Isolates; PPI = Pea Protein Isolates

Functional properties: The functional properties studied for cowpea, peas, pigeon pea and mungbean protein isolates are discussed below.

Bulk density: There existed significant variations in bulk density in different legumes protein isolates. The bulk densities were higher in cowpea and pea protein isolates i.e. 0.71±0.05 and 0.68±0.04 g/cm^{3}, respectively whilst pigeon pea and mungbean protein isolates behaved alike (Table 3). The defatting process results in porous texture of the defatted product that can be attributed for low bulk density would be an advantage in the formulation of complementary foods (Akpata and Akubor, 1999). Present results are supported by Akubor *et al.* (2003); the cowpea flour has bulk density 0.64 g/cm^{3}, while its isolates have bulk density of 0.82 g/cm^{3} as investigated by Ragab *et al.* (2004). Among the legume flours, peas and pigeon pea flours showed bulk density of 0.55 and 0.46 g/cm^{3}, respectively whereas, Kaur and Singh (2007) reported similar findings for chickpea and winged bean flours.

Protein solubility: Protein solubility is a useful indicator for the performance of protein isolates incorporated in the food systems and to determine the extent of protein

Table 3: Means for Bulk Density (BD), Protein Solubility (PS), Water and Oil Absorption Capacity (WAC&OAC) of protein isolates

Protein Isolates	BD (g/cm^3)	PS (%)	WAC (%)	OAC (%)
PPPI	0.53±0.02^b	68±3.09^{bc}	97±4.41^c	168±11.72^a
CPI	0.71±0.05^a	65±4.53^c	138±9.63^b	145±6.59^b
MBPI	0.55±0.03^b	72±4.44^b	163±10.05^a	113±6.84^c
PPI	0.68±0.04^a	82±4.97^a	152±9.20^a	140±8.63^b

denaturation because of heat or chemical treatment at different pH (Horax *et al*., 2004). Protein solubility of cowpea, peas, mungbean and pigeon pea at pH 7 were 65±4.53, 82±4.97, 72±4.44 and 68±3.09%, respectively (Table 3). The solubility of a protein is usually affected by its hydrophilicity or hydrophobic balance, depending on the amino acid composition, particularly at the protein surface (Moure *et al*., 2006). Higher solubility of pea protein isolates as compared to cowpea protein isolates may be due to the presence of low number of hydrophobic residues and the elevated charge. Results are comparable to the earlier findings of Sumner *et al*. (1980); they described 87% protein solubility for freeze dried pea protein isolates at neutral pH. Horax *et al*. (2004) reported the protein solubility of 80% for cowpea protein isolates at same pH. According to Mizubuti *et al*. (2000) pigeon pea protein solubility is more than 70%. Mwasaru *et al*. (2000) calculated 53.4 and 61.8% protein solubility for pigeon pea and cowpea, respectively. Afterwards, locust bean protein solubility was recorded upto 77% at pH 7 by Lawal (2004).

Water and oil absorption: Protein has both hydrophilic and hydrophobic properties thereby can interact with water and oil in foods. Results for water absorption revealed insignificant differences between mungbean (163±10.05%) and pea protein isolates (152±9.20%). Pigeon pea protein isolates showed lowest water absorption (97±4.41%) and highest oil absorption capacity (168±11.72%) whereas lowest oil absorption (113±6.84%) was observed in mungbean protein isolates (Table 3). Variation in water and oil absorption capacity of protein isolates may be due to different protein concentration, their degree of interaction with water and oil and possibly their conformational characteristics. The lower water absorption capacity of protein isolates is due to less availability of polar amino acids (Kuntz, 1971) and low fat absorption may be due to the presence of large proportion of hydrophilic groups and polar amino acids on the surface of the protein molecules (Sathe *et al*., 1982). Ragab *et al*. (2004) found that water and oil holding capacity for cowpea protein isolates are 220% and 110%, respectively. In case of pea protein isolates, Fernandez-Quintela *et al*. (1997); observed 170% water absorption capacity and 120% oil absorption capacity. According to El-Adawy (2000), mungbean had water and oil absorption capacity 200% and 135%, respectively and for pigeon pea 87% and 173% (Mizubuti *et al*., 2000). Also these findings are in line with those reported by others (Sefa-Dedeh and Yiadom- Farkye, 1988; Paredes-Lopez *et al*., 1991; Kaur and Singh, 2007).

Emulsifying activity and stability: Protein, being the surface active agents, can form and stabilize the emulsion by creating electrostatic repulsion on oil droplet surface (Makri *et al*., 2005). Maximum emulsifying activity was observed in pigeon pea protein isolates (49.50±3.00%) followed by cowpea (47.50±3.31%), peas (45.50±2.80%) and mungbean (41.10±1.87%) as indicated in Table 4. Significant differences occurred between legumes protein isolates regarding emulsion stability and mean values demonstrated higher stability (83.30±5.04%) in pigeon pea followed by 52.20±3.64 and 43.19±1.96% in cowpea and peas, respectively whilst, lowest 21.00±1.29% was observed in mungbean (Table 4). The results of the present study are in concordance with those reported earlier by Mwasaru *et al*. (2000); who calculated 48.16 and 54.90% emulsifying activity and stability for cowpea and 39.50 and 44.98% for pigeon pea protein isolates. According to Mizubuti *et al*. (2000) pigeon pea contained 97.97% emulsion stability. Ragab *et al*. (2004) reported the emulsifying activity (50%) and stability (82%) for cowpea protein isolates. Sumner *et al*. (1980) reported the emulsifying activity of freeze dried field pea protein isolates about 38%. Later, emulsion stability of 55.5% was observed by Fuhrmeister and Meuser (2003) for smooth pea protein isolates. The study conducted by El-Adawy (2000) showed that mungbean has emulsion activity about 65% and stability 18%. Afterwards, Lqari *et al*. (2002) reported the emulsion stability of 71% for lupin protein isolates.

Foaming capacity and stability: The foaming properties are used as indices of the whipping characteristics of protein isolates (Mwasaru *et al*., 1999). Maximum foaming capacity was observed in mungbean protein isolates (110±6.78%). However, that for cowpea and pigeon pea protein isolates exhibited no significant differences (Table 4). Whereas, means for different legumes demonstrated that peas showed significantly higher stability (79±4.78%) and lower (58±3.58%) was observed in mungbean protein isolates (Table 4). Low foaming capacity could be due to inadequate electrostatic repulsions, lesser solubility and hence excessive protein-protein interactions (Kinsella *et al*., 1985). Whereas, higher value for foaming stability

Table 4: Means for Emulsion Activity (EA) and Stability (ES), Foaming Capacity (FC) and Stability (FS) of protein isolates

Protein Isolates	EA (%)	ES (%)	FC (%)	FS (%)
PPPI	49.50±3.00[a]	83.30±5.04[a]	68±3.09[c]	71±3.23[b]
CPI	47.50±3.31[a]	52.20±3.64[b]	69±4.81[c]	65±4.53[b]
MBPI	41.10±1.87[b]	21.00±1.29[d]	110±6.78[a]	58±3.58[c]
PPI	45.50±2.80[a]	43.19±1.96[c]	78±4.72[b]	79±4.78[a]

Table 5: Least gelation concentration (LGC) of protein samples

Concentration (%)	PPPI	CPI	MBPI	PPI
2	(-)	(-)	(-)	(-)
4	(-)	(-)	(-)	(-)
6	(-)	(-)	(-)	(-)
8	(-)	(-)	(-)	(-)
10	(±)	(-)	(-)	(-)
12	(±)	(±)	(±)	(-)
14	(+)	(±)	(±)	(±)
16	(+)	(+)	(+)	(±)
18	(+)	(+)	(+)	(+)
20	(+)	(+)	(+)	(+)
LGC	14	16	16	18

indicates highly hydrated foams and decrease in foaming stability might be due to protein denaturation. Kaur and Singh (2007) observed decrease in foam volume with the passage of time for protein isolates of different chickpea cultivars. A similar trend has been reported for great northern bean proteins by Sathe and Solunkhe (1981) and for mucuna bean protein concentrates by Adebowale and Lawal (2003). The decrease in foam volume as a function of time was observed for all protein isolates. The results of the present investigation are in harmony with the finding of Mwasaru *et al.* (2000); they reported foaming capacity and stability for cowpea 35.30, 79.40% and 34.00, 77.80% for pigeon pea protein isolates, respectively. Ragab *et al.* (2004) reported foaming capacity of 65% for cowpea protein isolates. Pigeon pea had foaming capacity and stability 80, 102% as reported by Akintayo *et al.* (1999) and 44.70, 78.79% by Mizubuti *et al.* (2000), respectively. Foaming capacity 143% for freeze dried field pea protein isolates were determined by Sumner *et al.* (1980). Likewise, values for these traits in mungbean were 108 and 58% (El-Adawy, 2000). In a study conducted by Fernandez-Quintela *et al.* (1997), it was observed that pea protein isolate has 94% foaming stability.

Least gelation concentration: A qualitative parameter expresses the minimum protein concentration at which the gel does not slide along the test tube walls in inverted position (Moure *et al.*, 2006). The lower the least gelation concentration the better is the gelling ability of proteins (Akintayo *et al.*, 1999) because protein gels are aggregates of denatured molecules. Results showed that peas contained higher least gelation concentration (18%) followed by cowpea (16%) and mungbean (16%), whilst pigeon pea showed least gelation than others (14%) as obvious from Table 5. Fernandez-Quintela *et al.* (1997), reported 18% least gelation concentration for pea protein isolates; whereas 12% for cowpea protein isolates has been reported by Horax *et al.* (2004). Previously, Mwasaru *et al.* (2000) reported 14% LGC for pigeon pea and cowpea. Similarly, 16% least gelation concentration of cowpea protein isolates was observed by Onimawo and Akpojovwo (2006). According to Mizubuti *et al.* (2000); pigeon pea form gel at 12%. Circle and Smith (1972) reported that firm and resistant gels are formed from soy protein isolates at 16-17% concentrations.

Conclusion: In the developing countries there is an existing dilemma of protein energy malnutrition therefore some new indigenous sources must be exploited against the menace. Present investigation explicated the potential of various legumes for the preparation of protein isolates. Functional properties like water and oil absorption of protein isolates are suitable to be used further for the preparation of protein enriched products. Likewise bulk density, emulsion and foaming capacity and stability necessitate the use of such isolates in food system.

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