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Isolation and Partial Characterization of Chickpea, Lupine and Lentil Seed Proteins

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Abstract: Recently chickpea lupine and lentil seed proteins have been the focus of chemical and nutritional interest as a good substitute for soybean protein in the preparation of infant formulas and human foods. The purpose of the present investigation is to study the effect of different methods of protein isolation on the chemical and physical properties of the isolated proteins. Proteins were isolated in two steps: First protein was solubelized using alkaline conditions (pH 7-12) and with or without inorganic solutes (NaCl, Na_2SO_3 and $MgCl_2$). Second protein was precipitated from solution by using different techniques: Isoelectric point (pI), ammonium sulfate, methanol and ethanol). Optimum pH of chickpea protein solubilization was pH 11, but for lupine and lentil seed proteins pH was 12. Ammonium sulfate and alcohols precipitated all proteins. All proteins were deficient in sulfur amino acids but sufficient in acidic amino acids. Chickpea protein isolated by alcohols registered the highest water absorption, while that recovered by isoelectric point gave medium values. On the other hand, chickpea protein obtained with ammonium sulfate showed the minimum value. The same trend of water absorption was observed in lupine and lentil seed proteins. All proteins recovered by isoelectric point achieved the highest emulsion capacity. While proteins recovered by ammonium sulfate showed the highest emulsion stability. On the other hand, foaming capacity of lentil seed protein recovered by alcohols registered the highest values. Foaming stability of chickpea recovered by ammonium sulfate and ethyl alcohol was the highest while, foaming stability of lupine and lentil seed proteins recovered by ammonium sulfate was attained the highest values. This phenomenon is a function of the agent used for protein precipitation irrespective of the protein source.

Key words: Legume proteins · isolation · Biochemical and functional properties

INTRODUCTION

Edible legumes provide a readily available and economical source of proteins for much of the world's population. The protein-starved condition of the inhabitants of tropical Africa and other parts of the world, could be improved greatly by more widespread use of edible legumes, since they are an important source of the essential amino acids [1]. The nutritional value of legume seeds is limited by their low content of sulfur amino acids, particularly methionine [2]. There are two major types of storage proteins in legume seeds, vicilins and legumins, which are distinguishable by their sedimentation coefficients [3]. Legume proteins are utilized as meat protein substitute in some Frankfurter type sausages [4]. Recently chickpea, lupine and lentil seed proteins have been the focus of chemical and physical interest as a good substitute for soybean protein in the preparation of infant formulas and human foods. Apart from the highly acceptable taste of toasted chickpea its protein retains a reasonable nutritional value. However, amino acid composition reveals that the methionine is the first limiting amino acid [5]. Chickpea, Lupine and Lentil seeds contain 25-30% protein and are rich in essential and non-essential amino acids. Sánche-Vioque *et al.* [6] isolated Chickpea proteins using alkaline extraction followed by acid precipitation. They showed that the percentage of protein recovered from chickpea flour in the preparation of Isolates-A and B were 65.9 and 62.1%, respectively and had a balanced content of essential amino acids, with respect to the FAO pattern [6]. Extensive information is available on soy protein characteristics and functional

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properties. However, such information on chickpea protein is limited [7]. Supplementation of chickpea protein concentrate with methionine enhanced from 1.86 to 2.14 per value [8]. Roasted chickpea is consumed in Egypt as a favorite entertainment food. The Mediterranean diet has recently been rediscovered by nutritionists and doctors. Legumes, including chickpeas, are a main component on this healthy menu. Legume seeds contain three predominant storage proteins and one relatively minor storage protein [9]. Lentil and lupine are available sources of protein, with high protein content [10] but not studied as extensively as a common beans and soybeans. Legume seed proteins are, on a global scale, an important food protein resource. The major obstacle in the study of legume seed proteins has been the difficulty encountered in obtaining homogeneous preparations of these proteins [11]. Legume proteins are thought to be beneficial as an anti-diabetic, low glycaemic index food and rich in antioxidants. Evidence suggests that these three novel sources of legumes may provide health benefits when included in the daily diet [12]. Native lupine proteins (alpha, beta and gamma) conglutin have a good solubility [13]. Lupine proteins were high in lysine but relatively low in sulfur-containing amino acids. Good solubility and moderate emulsifying, foaming and gel-forming properties of isolates were observed [14]. Functional potential was introduced to better approach to the understanding of the relationships between the structure and the functional properties of food proteins [15]. Chickpeas, lentils and Lupines are among the select group of foods that provide protein as well as calcium and iron. Chickpea proteins have a good nutritional quality and could be incorporated in food systems, but their functional properties have not been extensively determined to utilize these valuable sources of protein for food applications. More information on the chemical and functional properties is needed. The chemical and biochemical studies of lentil and lupine seed proteins are mostly concerned with protein content, amino acid composition, water and oil absorption as well as emulsion capacity and stability. Hence, the aim of the present study was to compare different protein products extracted from chickpea, lupine and lentil seeds using various methods for extraction and precipitation. This approach will help the manufacturer and to select the most optimal procedure and conditions for producing protein concentrates or isolates having the best chemical and physical properties and economize the costs of production.

MATERIALS AND METHODS

Chickpea (*Cicer aritinium*), Lupine (*Lupinus mutabilis*) and lentil (*Lens culinaris*) were obtained from Ministry of Agriculture (Cairo, Egypt). Chemicals and reagents were obtained from BDH Ltd. Pharmacia and BioRad. The samples were ground by grinder (BROUN-MULTIQUICK SYSTEM-ZK100) until became fine powder. Total protein and moisture were determined according to the methods described in A.O.A.C. [16]. Total lipids were extracted by chloroform and methanol (2:1v/v) Folsh [17].

Protein extraction: Chickpea, lupine and lentil seed proteins were solubilized [6, 17] using fifty grams of defatted chickpea seed suspended in one liter distilled water (Dist H_2O, 25°C, pH 7). The pH was adjusted with 0.1M HCl or 0.1M NaOH (pH values used were 6, 7, 8, 9, 10, 11 and 12). The pH was maintained for 60 min at room temperature with agitation (final concentration 1:20 w/v). The suspension was centrifuged at 6000 rpm, 20°C for 30 min and the supernatants were stored at 4°C until use. Protein content in the supernatant was measured spectrophotometrically at 545 nm [18] using the following equations:

$$\% \text{ protein conc.} = \frac{\text{A sample}}{\text{A st.}} \times n$$

$$n = 10 \text{ g}/100 \text{ ml}, \quad n = 100 \text{ g l}^{-1}$$

The solubility profile of seed proteins was also determined by similar process in the presence of NaCl, Na_2SO_3 and $MgCl_2$ at different molar concentrations (0.1-0.6 M) at a fixed pH = 8.

Protein precipitation:

Acid precipitation: Extracted proteins were precipitated at different pHs (3, 3.5, 4, 4.5, 5 and 5.5) [6, 19] with 0.1 M HCl. The precipitate was recovered by centrifugation at 7000 rpm for 30 min at 10°C. The precipitates were washed twice with distilled water at the same pH and then centrifuged and freeze dried.

Ammonium sulfate precipitation: Protein precipitation by ammonium sulfate was carried out [18]. Protein solution was placed in a beaker kept in ice and put over a magnetic stirrer (stirring was started slowly) then 61.2 g of solid ammonium sulfate were added per 100 ml of solution. The pH of the solution was adjusted with 0.1 M NaOH.

The sample was centrifuged for 15 min at 7000 rpm and the supernatant was decanted off. Protein pellets were resuspended in the desired volume of buffer and residual ammonium sulfate was removed by dialysis.

Alcoholic precipitation: Alcoholic precipitation of proteins was carried out [20]. One volume of cold (-20°C) 95% ethanol or methanol was added to the same volume (100 ml) of protein solution and left for at least 30 min. The mixture was kept cold and stirred gently. This was accomplished by providing an ice water jacket then the suspension was allowed to stir for an additional 15 min. The resulting precipitate was centrifuged for 20 min at 15000 rpm and the supernatant was carefully decanted while the residue was dried under vacuum.

Amino acid determination: Amino acid analysis was carried out using performance amino acid analyzer (Beckman 7300) [21].

Functional properties: Oil and water absorption, emulsion capacity and stability were carried out as described by Sathe and Salunkhi [22].

RESULTS

Results obtained in Table 1 showed that total protein content of chickpea, lupine and lentil was 25, 23 and 21.5%, respectively. Total lipids were 4.5, 9 and 7.5% and moisture was 9.7, 11 and 13%, respectively.

Protein extraction: Protein extraction is normally governed by the pH values, which influence the ratio of free to neutralized charges. The data of the present study declared that protein solubility was gradually enhanced with the increase in pH values. However, maximum solubility of chickpea protein (80%) was obtained at pH 11 and those of lupine and lentil seed proteins (83 and 87%, respectively) were achieved at pH 12 (Tables 1-3). The data in (Tables 1-3) represent the results of adding certain salts to the dissolving medium at the optimal pH. It can be generally observed that non-of the salts added had any favorable effect on the solubility of chickpea, lupine and lentil seed proteins. Monovalent ions (Na^+) did not increase protein solubility at all concentrations used (0.1-0.6 M), more precisely, Monovalent ions slightly reduced protein solubilization. On the other hand, bivalent cations (Mg^{++}) had a reducing effect on the protein solubilization at all concentrations used (0.1-0.6 M).

Table 1: Solubility percentages of chickpea proteins with alkaline solutions (pH 11) at different salt concentrations

NaCl pH 11	Protein solubility %	Na_2SO_3 pH 11	Protein solubility %	$MgCl_2$ pH 11	Protein solubility %
0.0M	80.0	0.0M	80.0	0.0M	80.0
0.1M	68.0	0.1M	80.0	0.1M	10.8
0.2M	78.5	0.2M	78.5	0.2M	15.0
0.3M	73.0	0.3M	77.6	0.3M	17.6
0.4M	70.5	0.4M	74.7	0.4M	16.0
0.5M	78.2	0.5M	72.0	0.5M	16.3
0.6M	62.2	0.6M	67.0	0.6M	15.0

Table 2: Solubility percentages of lupine seed proteins with alkaline solutions (pH 12) at different salt concentrations

NaCl pH 12	Protein solubility %	Na_2SO_3 pH 12	Protein solubility %	$MgCl_2$ pH 12	Protein solubility %
0.0M	83.0	0.0M	83.0	0.0M	83.0
0.1M	47.0	0.1M	92.0	0.1M	80.0
0.2M	90.0	0.2M	89.0	0.2M	40.0
0.3M	86.0	0.3M	86.0	0.3M	57.0
0.4M	83.0	0.4M	81.0	0.4M	52.0
0.5M	72.0	0.5M	68.0	0.5M	23.0
0.6M	85.0	0.6M	53.0	0.6M	81.0

Table 3: Solubility of lentil seed protein with alkaline aqueous solutions pH (12) in the presence of different salt concentrations

NaCl pH 12	Protein solubility %	Na_2SO_3 pH 12	Protein solubility %	$MgCl_2$ pH 12	Protein solubility %
0.0M	87.5	0.0M	87.5	0.0M	87.5
0.1M	85.7	0.1M	82.5	0.1M	32.5
0.2M	89.0	0.2M	80.1	0.2M	60.6
0.3M	83.9	0.3M	85.8	0.3M	34.3
0.4M	73.5	0.4M	70.5	0.4M	48.5
0.5M	71.5	0.5M	60.0	0.5M	50.0
0.6M	53.0	0.6M	54.5	0.6M	42.5

Protein precipitation:

Isoelectric point: Proteins solubilized at optimum pH values were precipitated at pHs ranging from 3 to 5.5. Hence, there is a common major protein component in three protein materials whose isoelectric point is around pH 4.5 (Table 4). The highest yield of precipitated protein was obtained at pH 4.5. The highest precipitation was recorded with lupine seed protein (87.30%) followed by chickpea protein (81.4%) and finally lentil seed protein (80%) (Tables 4 & 5).

Table 4: Protein precipitation at different acidic pH values

	Protein recovery (%)		
pH	Chickpea	Lupine	Lentil
3.00	55.80	79.90	74.50
3.50	75.90	80.20	62.30
4.00	79.80	86.40	78.00
4.50	81.40	87.30	80.00
5.00	75.40	84.10	74.50
5.50	73.60	81.10	71.60

Table 5: Protein precipitation using different agents

Precipitating agent	Chickpea	Lupine	Lentil
pI	81.40	87.30	80.00
$(NH_4)_2SO_4$	90.60	92.60	93.00
CH_3OH	97.80	100.00	100.00
C_2H_5OH	100.00	100.00	100.00

Ammonium sulfate precipitation: Results showed that all proteins have been precipitated by ammonium sulfate to high extents (90-93%) (Table 5). The highest precipitation was recorded with lentil seed proteins (93%) followed by lupine seed proteins (92.6%) then chickpea proteins (90.6%).

Alcoholic precipitation: The proteins have been precipitated from their alkaline solutions by adding methyl or ethyl alcohol at a ratio of 1:1 v/v (Table 5). The precipitation recorded highest level of 100% referring to the precipitation of all protein components irrespective of their chemical characteristics [7]. This phenomenon exclusively observed with organic solvent precipitation which is attributed to the potential of alcohols to reduce water availability to a minimum level rending all protein components insoluble. Then it may be understood that alcohol has precipitated all protein components irrespective of molecular sizes (Table 5).

Amino acid composition: From the obtained results it can be observed that all proteins contained of high levels of acidic amino acids (Aspartic and Glutamic acids) (Tables 6-8). The highest acidic amino acids recoveries were associated with ammonium sulfate as a precipitating agent. On the other hand, the three protein samples seemed to be poor in sulfur amino acids (Cystine and Methionine). This result was observed in chickpea protein recovered by pI, $(NH_4)_2SO_4$, CH_3OH and C_2H_5OH. The sulfur containing amino acids were found in a high values in protein samples precipitated by acid. The basic amino acids (lysine, arginine and histidine) showed also high recovery levels especially with proteins precipitated by ammonium sulfate or alcohol in all samples. This trend holds true especially for lysine which is greatly important to the nutritional value of legume proteins. Acid precipitation might have negative effect in the recovery of the basic amino acids probably due to the chemical effects of the acidic agent on the basic amino acids. The change in the other amino acids was trivial and did not give a remarkable trend.

Functional properties:

Water and oil absorption: The present study showed that the chickpea protein isolate which recovered by alcohols registered the highest water absorption, while protein recovered by pI gave medium values. On the other hand, chickpea protein isolate showed the minimum water absorption value (about 21%) which obtained by ammonium sulfate precipitation (Table 9). The same trend (water absorption) was noticed for lupine and lentil seed proteins. This let us say that water absorption by precipitated protein is a function of the reagent used for precipitation irrespective of the protein source. This unique phenomenon associated with the products obtained by ammonium sulfate precipitation might refer to the mechanism by which protein was precipitated.

Regarding the oil absorption of proteins it is evident that all protein isolates recovered by ammonium sulfate gave also the least values for oil absorption (Table 9). On the other hand, all proteins obtained by isoelectric point precipitation exhibited the highest values of oil absorption.

Emulsion capacity and stability: The emulsion capacity of chickpea protein isolates recovered by pI, ammonium sulfate, methanol and ethanol were 66.80, 35.00, 48.500 and 57.00 g g^{-1}, respectively. It is evident that the emulsion capacity of chickpea protein isolate recovered by pI recorded the highest value. On the other side, chickpea protein isolate recovered by ammonium sulfate recorded the lowest value. However, the protein recovered by ammonium sulfate which achieved the lowest emulsion capacity exhibited the highest emulsion stability. Similarly, the emulsion capacity of lupine seed recovered by pI, ammonium sulfate and alcohols were 65.60, 43.00, 59.00 and 55.00 g g^{-1}, respectively. It can be observed that the protein recovered by isoelectric point (pI) recorded the highest emulsion capacity and more stable than that recovered by ammonium sulfate and alcohols. Likewise, the emulsion capacity of lentil seed protein recovered by pI, ammonium sulfate, methyl and ethyl

Table 6: Amino acids composition of Chickpea seed protein precipitates with pI, $(NH4)_2SO_4$, CH_3OH and C_2H_5OH.

Amino acid	pI (%)	$(NH_4)_2SO_4$ (%)	CH_3OH (%)	C_2H_5OH (%)	Amino acid	pI (%)	$(NH_4)_2SO_4$ (%)	CH_3OH (%)	C_2H_5OH (%)
Aspartic acid	10.00	11.60	10.50	9.55	Methionine	0.64	0.37	-	0.48
Threonine	1.41	2.28	2.52	2.85	Isoleucine	3.52	4.14	3.61	3.62
Serine	1.40	2.44	2.32	3.45	Leucine	6.54	8.00	6.50	6.07
Glutamic acid	17.00	18.00	17.00	16.50	Tyrosine	2.63	3.31	3.00	1.97
Proline	3.45	4.10	3.58	3.41	Phenylalanine	4.04	4.86	4.10	3.42
Glycine	3.09	3.52	3.58	3.70	Histidine	2.03	2.30	2.10	2.17
Alanine	3.22	3.83	4.04	3.77	Lysine	5.53	6.20	6.20	5.92
Cystine	1.90	0.82	1.32	0.24	Arginine	5.81	6.37	6.02	6.35
Valine	3.74	4.34	4.05	4.00	Total	75.95	86.50	80.44	77.47

Table 7: Amino acids composition of Lupine seed proteins precipitated with pI, $(NH4)_2SO_4$, CH_3OH and C_2H_5OH

Amino acid	pI (%)	$(NH_4)_2SO_4$ (%)	CH_3OH (%)	C_2H_5OH (%)	Amino acid	pI (%)	$(NH_4)_2SO_4$ (%)	CH_3OH (%)	C_2H_5OH (%)
Aspartic acid	9.80	10.10	11.40	6.23	Methionine	-	-	-	-
Threonine	1.50	3.13	2.68	1.66	Isoleucine	4.11	4.34	3.35	2.32
Serine	1.31	4.00	4.07	2.00	Leucine	6.70	6.70	6.40	4.10
Glutamic acid	19.00	18.30	18.00	15.11	Tyrosine	4.43	5.40	2.40	2.40
Proline	3.96	4.10	3.17	2.19	Phenylalanine	3.73	4.00	4.40	2.00
Glycine	3.34	3.27	3.91	2.18	Histidine	1.70	2.00	2.18	1.15
Alanine	3.10	3.00	3.81	2.00	Lysine	3.75	4.00	6.25	2.20
Cystine	1.70	0.52	0.56	0.34	Arginine	6.54	6.71	9.56	4.50
Valine	3.57	3.70	5.00	2.12	Total	78.24	83.27	87.14	52.50

Table 8 : Amino acids composition of Lentil seed proteins precipitated with pI, $(NH4)_2SO_4$, CH_3OH and C_2H_5OH

Amino acid	pI (%)	$(NH_4)_2SO_4$ (%)	CH_3OH (%)	C_2H_5OH (%)	Amino acid	pI (%)	$(NH_4)_2SO_4$ (%)	CH_3OH (%)	C_2H_5OH (%)
Aspartic acid	9.32	12.34	10.25	10.18	Methionine	-	-	0.34	0.25
Threonine	2.68	2.78	2.64	2.48	Isoleucine	3.55	4.08	3.76	3.65
Serine	3.87	3.50	4.22	3.07	Leucine	6.23	7.75	7.06	6.81
Glutamic acid	14.50	18.10	19.33	16.81	Tyrosine	2.55	3.35	2.65	2.95
Proline	3.26	3.85	3.60	2.19	Phenylalanine	4.30	7.09	5.09	6.05
Glycine	2.78	3.60	3.85	2.12	Histidine	1.76	2.16	1.86	1.86
Alanine	3.00	3.75	4.00	2.83	Lysine	5.00	6.43	5.23	1.17
Cystine	0.12	0.74	0.20	0.14	Arginine	5.08	8.33	7.88	6.88
Valine	3.81	5.42	4.71	4.71	Total	71.81	92.60	90.67	74.15

Table 9: Water and oil absorption capacity of chickpea, lupine and lentil seed proteins

	Water and oil absorbed (ml g^{-1})					
	Chickpea protein		Lupine protein		Lentil protein	
pH	water	Oil	water	Oil	water	Oil
pI	4.00	3.52	3.70	3.52	3.50	3.96
$(NH_4)SO_4$	1.20	1.10	0.90	2.00	1.00	2.00
CH_3OH	5.60	2.00	4.30	2.80	4.50	2.30
C_2H_5OH	5.80	2.40	3.80	2.00	3.90	1.80

alcohols were 65.00, 35.00, 55.00 and 60.00 g g^{-1}, respectively. The emulsion capacity of protein recovered by pI attained similarly the highest level, while that recovered by ammonium sulfate recorded the lowest value. The lentil seed proteins recovered by ammonium sulfate and pI showed also the highest emulsification stability.

DISCUSSION

In this study, the difference in solubility behavior between chickpea (pH 12), lupine and lentil (pH 11) may be due to the difference in their amino acid composition. All studied proteins showed high solubility at the alkaline pH values as a result of their high content of the acidic amino acids which tends to be ionized at this pH range. These differences may be due to different associations

and complexes between protein component and other constitutes in each plant materials. On the other hand, isoelectric point of major protein components in three protein materials (Chickpea, lupine and Lentil) around pH 4.5, but ammonium sulfate is a good precipitating agent for major protein content from three protein sources. The high efficiency precipitation which observed with organic solvents is attributed to the potential of alcohols to reduce water availability to minimum levels rending all protein components insoluble. This may understood that alcoholic precipitation has precipitated all protein components irrespective of molecular sizes (Table 5).

The total recovery of amino acids was lower with acid precipitation than ammonium sulfate or alcoholic precipitation, which may refer to some chemical interactions between the precipitating agent and the protein component.

In water absorption the study showed that, the chickpea lupine and lentil protein isolates recovered by alcohols gave the highest water absorption, while protein recovered by pI gave medium values. In the same time, protein isolates showed the minimum water absorption value for protein obtained by ammonium sulfate precipitation (Table 9). This is due to a function of the agents used for protein precipitation irrespective of the protein sources and the unique phenomenon associated with the products obtained by ammonium sulfate precipitation might refer to the mechanism by which protein was precipitated. Ammonium sulfate might reduce the availability of water to minimum level and hence, pushing protein molecules to precipitate by interacting and aggregating with each other. This might have concealed the polar residual groups and prevented them from interacting with water molecules in water absorption test. Oil absorption of proteins was evident that, all protein isolates recovered by ammonium sulfate gave also the least values for oil absorption (Table 9). This may confirm the previous conclusion which means that the precipitated protein molecules were aggregated in away giving low access either to water or oil molecules. On the other hand, all proteins obtained by isoelectric point precipitation exhibited the highest values of oil absorption. This may be evidently due to the fact that all the acidic or basic amino acid residues were neutralized though this technique of precipitation and consequently the polarity of recovered proteins were less than those obtained by other means. Hence, these proteins showed more affinity to oil than those prepared by alcohol and ammonium sulfate precipitation.

In emulsion capacity and stability behavior for all protein isolates it can be concluded that all protein isolates recovered by pI precipitation achieved the highest emulsion capacity irrespective of the protein source. The protein isolates recovered by ammonium sulfate and alcohols, however, showed the highest emulsion stability. This may be due to that protein isolate recovered by pI is characterized by neutralized side chain groups which might have encouraged the interaction between protein molecules and oil phase. While the protein recovered by ammonium sulfate and alcohols which maintained all charged groups in their original sates was less interactive with the oil phase.

CONCLUSIONS

The purpose of the present study is to study the effect of different methods of protein isolation on the chemical and functional properties. Protein was isolated in two steps. The first step was protein solubilization using alkaline conditions (pH 7-12) and adding organic solutes. The second step was protein precipitation from protein solution by using different techniques. The results pointed out that the optimum pH of protein solubilization was pH 11 for chickpea protein solubilization and pH 12 for lupine and lentil seed proteins. On the other hand, inorganic solutes did not enhance protein solubility at the optimum pH of solubilization. Ammonium sulfate and alcohols precipitated all the proteins from protein solution while, isoelectric point precipitation achieved the least protein recovery. All protein samples were deficient in sulfur amino acids regardless of the method of protein isolation or protein source. But these were sufficient in acidic amino acids in all protein samples. All proteins obtained by pI exhibited the highest values of oil absorption, while, all proteins recovered by ammonium sulfate gave the least values for water and oil absorption. All protein isolates recovered by pI precipitation achieved the highest emulsion and foaming capacity irrespective of the protein source, while the protein isolates recovered by ammonium sulfate and alcohols showed the highest emulsion and foaming stability. For food and supplementation and nutrition purposes we recommend to use the ammonium sulfate and alcoholic as precipitating agent for legume seed protein isolation. These agents reserve the chemical and functional properties of protein isolates. I n the same time these isolation methods cheap and easy to use.

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