ELSEVIER

Food Chemistry 64 (1999) 237–243

Food Chemistry

Protein isolates from chickpea (*Cicer arietinum* L.): chemical composition, functional properties and protein characterization

R. Sánchez-Vioque [a], A. Clemente [a], J. Vioque [a], J. Bautista [b], F. Millán [a,*]

[a] *Departamento de Fisiología y Tecnología de Productos Vegetales, Instituto de la Grasa (C.S.I.C.), Avda. Padre Garcia Tejero, 4, 41012, Sevilla, Spain*
[b] *Departamento de Bioquímica, Bromatología y Toxicología, Facultad de Farmacia, Universidad de Sevilla, Sevilla, Spain*

Received 13 January 1998; received in revised form 28 May 1998; accepted 8 June 1998

Abstract

Two types of protein isolates were prepared from ground chickpea seeds by alkaline extraction, with (Isolate-B) and without (Isolate-A) sodium sulphite, and acid precipitation of the proteins at the isoelectric point (pI 4.3). The percentage of protein recovered from chickpea flour in the preparation of Isolates-A and B were 65.9 and 62.1%, respectively. Chemical composition, main functional properties and protein composition of chickpea flour and protein isolates were determined. Isolates-A and B contained 78 and 88.1% of protein, respectively, and had a balanced content of essential amino acids, with respect to the FAO pattern. The *in vitro* protein digestibility ranged between 95.6 and 96.1%. Isolate-A showed a partial dissociation of the 11S protein because of the high pH used for the protein extraction, and this probably explains the differences observed in the functional characteristics of both isolates.

Keywords: Chickpea; Protein isolates; Chemical composition; Functional properties

1. Introduction

The production of plant protein isolates is of growing interest to industry because of the increasing application of plant proteins in food and non-food markets. In this context, the European Union is attempting to develop its own protein crops to decrease commercial dependence on protein availability (Chominot, 1992). The use of plant protein isolates in foods as functional ingredients, to improve the nutritional quality of the product or for economic reasons is very extended. Nevertheless, these applications in the food trade are almost limited to proteins from soybean seeds, whereas other vegetable proteins are less used. Among these are those from chickpeas (*Cicer arietinum* L.), that are extensively grown in different parts of the world and, in particular, in the Mediterranean Region (De Miguel Gordillo, 1991).

The alkaline extraction and subsequent precipitation of the proteins at the isoelectric point is the most usual way to prepare protein isolates in the food industry. The low cost of the chemical products and the relative simplicity of the apparatus required, make this option advantageous as compared to other procedures such as the separation of proteins by ultrafiltration membranes or the precipitation of salt soluble proteins by aqueous dilution (Berot & Davin, 1996).

Chickpea seeds are usually used for human consumption, but an important percentage of the production, about 20%, is damaged during the harvest and processing, and considered as a by-product that is sold at low prices for livestock feeding (Ulloa, Valencia, & Garcia, 1988). These seeds may be an interesting raw material for the production of protein isolates.

We have prepared two types of protein isolates from chickpea seeds, using sodium sulphite in the extraction medium or without it. The yield of the production, chemical composition, functional properties and protein composition of these isolates have been investigated in relation to the possible use of the protein isolates in the food industry.

2. Materials and methods

2.1. Materials

Chickpea seeds (c.v. *Athenas*) were a gift from Koipesol Semillas, S.A. (Sevilla, Spain). The seeds were ground and extracted with hexane in a soxhlet extractor for 9 h

*Corresponding author. Tel.: +34-5-4611550; Fax: +34-5-4616790; e-mail: frmillan@cica.es

0308-8146/98/$—see front matter
PII: S0308-8146(98)00133-2

to remove most of the fat. The resulting defatted chickpea flour was used as the starting material. Trypsin (EC 3.4.21.4), α-Chymotrypsin (EC 3.4.21.1), peptidase and D,L-α-aminobutyric acid were purchased from Sigma (St. Louis, Missouri, USA). Blue dextran 2000, thyroglobulin, β-amylase, bovine serum albumin and ribonuclease A were obtained from Pharmacia Biotechnology (Uppsala, Sweden). Coomassie Brilliant Blue G 250 and diethyl ethoxymethylenemalonate were purchased from Serva (Heidelberg, Germany) and Fluka (Buchs, Switzerland), respectively. All other chemicals were of analytical grade.

2.2. Analytical methods

Moisture, ash and nitrogen contents were determined using AOAC, (1990) approved methods. Total fibre was determined according to the procedure described by Lee, Prosky, and De Vries, (1992). Lipids associated to the chickpea flour and protein isolates were extracted following the method of Nash, Eldridge, and Wolf, (1967), and nonlipid material removed (Singh & Privett, 1970). Soluble sugars and polyphenols were measured using standard curves of glucose (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and chlorogenic acid (Moores, Mc Dermott, & Wood, 1948). Dissolved solids were determined according to Pomenta & Burns, (1971). Protein solubility was measured in a 0.1 M NaCl pH 7.0 solution (Morr, German, Kinsella, Regenstein, Van Buren, Kilara, Lewis, & Mangino, 1985). Water absorption was determined following Sosulski, (1962) procedure. Fat absorption and emulsion capacity were evaluated using the methods described by Lin, Humbert, & Sosulski, (1974).

2.3. In vitro protein digestibility

Samples containing 62.5 mg of protein were suspended in 10 ml of water and the pH adjusted to 8. An enzymatic solution containing 1.6 mg of trypsin (17.7 BAEE U mg^{-1}), 3.1 mg of α-chymotrypsin (43 U mg^{-1}) and 1.3 mg of peptidase (50 U g^{-}) per ml was added to the protein suspension in a 1:10 v/v ratio. The pH of the mixture was measured after 10 min and the in vitro digestibility calculated as a percentage of digestible protein using the equation: digestible protein = 210.464–18.103 × pH (Hsu, Vavak, Satterlee, & Miller, 1977).

2.4. Nonprotein nitrogen

For the determination of nonprotein nitrogen, 1 g of chickpea flour was extracted by stirring with 40 ml of 70% aqueous ethanol for 1 h. The slurry was centrifuged at 8000 × ***g***, and the supernatant recovered for the determination of nitrogen content (Bhatty, Sosulski, & Wu, 1973).

2.5. Determination of the isoelectric point (pI)

For the determination of the pI, 15 g of chickpea flour were extracted twice with 300 ml of 0.2% NaOH solution and centrifuged for 20 min as above. Aliquots (40 ml) of the supernatant were titrated with 0.5 N HCl to various pH values, ranging from 2.5 to 6.5. The precipitate formed was separated by centrifugation as above. The percentages of nitrogen in the supernatants in relation to the total nitrogen extracted were plotted vs. pH to determine the pI (see Fig. 1).

2.6. Preparation of protein isolates

2.6.1. Isolate-A

Chickpea flour (20 g) was suspended in 200 ml of 0.2% NaOH solution pH 12, and extracted by stirring for 1 h. After centrifugation at 8000 × ***g***, two additional extractions were carried out with half of the volume of alkaline solution. The supernatants were pooled and analyzed for nitrogen content. The pellet was dried in an oven at 50°C, weighed and analyzed for nitrogen content. The pH of the soluble proteins was adjusted to the isoelectric point (pH 4.3) and the precipitate formed was recovered by centrifugation as above. The precipitate was washed with distilled water adjusted to pH 4.3 and freeze-dried.

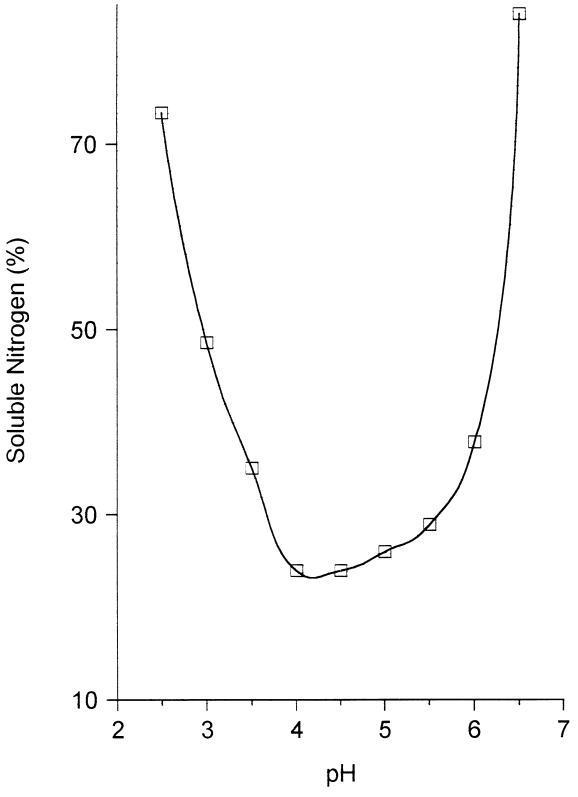

Fig. 1. Solubility curve for chickpea proteins. Solubility of the proteins is expressed by measuring the relative percentages of nitrogen soluble at various pH values.

2.6.2. Isolate-B

Chickpea flour (20 g) was extracted as above but with 0.25% Na_2SO_3 at pH 10.5 to avoid the darkening of the final product (Gheyasuddin, Cater, & Mattil, 1970). The precipitate obtained at the isoelectric point was successively washed with 100 ml of distilled water adjusted to pH 4.3, ethanol and acetone, and dried at room temperature.

2.7. Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using 0.75 mm thick gels (Laemmli, 1970). Acrylamide concentrations of stacking and running gels were 4 and 20%, respectively. The electrophoresis was run at 20 mA and gels were stained with Coomassie Brilliant Blue G 250. Samples for electrophoresis were prepared as follows: lyophilized samples (1 g) were dissolved in 10 ml of 0.1 M sodium borate, 0.2 M sodium chloride buffer pH 8.3 and eluted with the same buffer in a Sephadex G-25 column to remove nonprotein components. Purified extracts were diluted to 2 mg of protein per ml and mixed (1:1 v/v) with a solubilization buffer TRIS–HCl 80 mM, 0.57% EDTA, 0.26% DTT, 3.3% SDS, 0.008% Blue Bromophenol, 20% sucrose pH 6.8 and reduced with 2-mercaptoethanol in boiling water. Molecular masses were determined using the low molecular weight standards from Pharmacia LKB Biotechnology.

2.8. Gel filtration

Lyophilized samples (1 g) were dissolved in 10 ml of 0.1 M sodium borate, 0.2 M sodium chloride buffer pH 8.3 and purified in a Sephadex G-25 column as above. Gel filtration was carried out in a FPLC system equipped with a Superose 6 HR 10/30 column from Pharmacia LKB Biotechnology. Volume injection and concentration of the samples were 200 μl and 1.6 mg of protein per ml, respectively. The eluent was the above mentioned borate buffer at a flow rate of 0.4 ml min^{-1}. Elution was monitored at 280 nm. The approximate molecular masses were determined using blue dextran 2000 (2000 kDa), thyroglobulin (669 kDa), β-amylase (200 kDa), bovine serum albumin (67 kDa) and ribonuclease A (13.7 kDa) as molecular weight standards.

2.9. Amino acid analysis

Samples containing 2 mg of protein were hydrolyzed with 6 N HCl at 110°C for 24 h and derivatized with diethyl ethoxymethylenemalonate. Amino acids were determined by reversed-phase high-performance liquid chromatography (HPLC) using D,L-α-aminobutyric acid as internal standard (Alaiz, Navarro, Giron, & Vioque, 1992). The HPLC apparatus (Waters) consisted of a Model 600E multi-solvent delivery system, a Wisp Model 712 automatic injector and a 484 UV-Vis detector. Data acquisition and processing were effected with Maxima 820 3.3 version software (Waters). Separations were attained with a 300×3.9 mm i.d. reversed phase column (Nova Pack C_{18}, 4 μm, Waters) using a binary gradient system with 25 mM sodium acetate pH 6.0 and acetonitrile. The column was maintained at 18°C by a temperature controller (Julabo F 10). Amino acid composition was expressed as grams of amino acid per 100 g of protein.

3. Results and discussion

3.1. Preparation of chickpea protein isolates

Chickpea protein quality is equivalent to that of soybean meal (Friedman, 1996). Nevertheless, the chickpea seeds contain several antinutritional factors that decrease the protein utilization. According to Singh, (1988), proteases and amylase inhibitors, lectins, polyphenols and certain sugars are the main antinutritional factors of chickpea seeds. Other antinutritional factors found in chickpea seeds are the phytic acid and undigestible carbohydrates as raffinose and stachyose. Most of these compounds inhibit the digestive enzymes or react with essential amino acids limiting the application of the whole seed in many food products. This problem could be overcome if the chickpea proteins are isolated.

Isolate-A is easier and cheaper to prepare than Isolate-B because the former is obtained by extraction of the proteins without sodium sulfite, and does not use either ethanol or acetone for washing. However, Isolate-B has several advantages: extraction conditions are milder (pH 10.5 versus pH 12), and sodium sulphite inhibits polyphenol oxidation avoiding the subsequent reaction between proteins and oxidized polyphenols (Cheftel, Cuq, & Lorient, 1989). This reaction is responsible for the light brown colour of Isolate- A compared to the white colour of Isolate-B.

Solubility of chickpea proteins was minimum at pH 4.3 (Fig. 1). The percentage of protein extracted from chickpea flour, by a three step extraction process, was 80.9% for Isolate-A and 87.1% for Isolate-B. Nevertheless, the precipitation at the pI of the extracted proteins led to losses of those that are soluble at pH 4.3. These soluble proteins are especially albumins (Berot & Davin, 1996). The final protein recovered from chickpea flour was 65.9 and 62.1% in the Isolate-A and B, respectively.

3.2. Chemical composition of chickpea protein isolates

As a result of the ethanol and acetone washes, Isolate-B had a minor content of fibre, associated lipids, dissolved solids, soluble sugars and carbohydrates and

thereby its protein content (88.1%) was higher than in Isolate-A (78.0%) (Table 1). Although the chickpea flour was extracted with hexane, lipids were not removed completely and part of them (1.5%) remained in the flour and were associated with the protein isolates (Sánchez-Vioque, Clemente, Vioque, Bautista, & Millán, 1998a). These lipids, mainly of a polar nature (Sánchez-Vioque, Clemente, Vioque, Bautista, & Millán, 1998b), play an important role in flavor (Rackis, Sessa, & Honing, 1979) and in the interaction with proteins (Kikugawa, Ido, & Mikami, 1981). In this sense, Isolate-B should be less affected by these alterations because of its lower lipid content (1.1%) respect to Isolate-A (3.5%). The low polyphenol content, less than 0.1%, of chickpea flour and protein isolates is also noteworthy. Polyphenols react with proteins yielding dark protein isolates and decreasing the bioavailability of several amino acids. Low levels of polyphenols are therefore desirable.

3.3. Functional properties of chickpea protein isolates

Since potential uses of protein isolates are largely dependent on functional properties, the main physicochemical properties of the isolates were investigated (Table 2). Marked differences in protein solubility, water and fat absorption and emulsion capacity were observed between Isolate-A and B. Isolate-A showed a higher water and fat absorption than Isolate-B whereas this latter was more soluble and had a better emulsion capacity. Solubility of Isolate-B was 46.3%, almost two fold higher than that of Isolate-A (26.6%). This is probably due to the different conditions used for the extraction of the proteins. Isolate-B, that is prepared in the presence of the antioxidant sodium sulphite and at pH 10.5, may maintain the native conformation of the proteins to a larger extent than Isolate-A, that is prepared using more drastic conditions. This higher content of denatured proteins in Isolate-A than in Isolate-B would be reflected in a poor solubility. Also, the higher content of lipids associated to the protein Isolate-A may decrease its solubility. Likewise, emulsion capacity, that is largely dependent on the protein solubility (Cheftel et al., 1989), was lower in Isolate- A (48.1%) than in B (76.9%). The higher water absorption in Isolate-A (343.7 g) than in Isolate-B (199.5 g) could be due to the lower losses of soluble proteins in the former. Water absorption of Isolate-B was poor as compared with the values observed in soy isolates that generally swell up to 3–4 times their weight (Waggle, Steinke, & Shen, 1989). Isolate-A, because of its higher fat absorption (409.4 g), was more appropriate than B (125.7 g) to be used in foods in which fat retention is desirable, such as meat and dairy products.

Table 1
Chemical composition of chickpea flour (CF) and Isolate-A (IA) and B (IB). Data expressed as g 100 g^{-1} of dry matter are the mean ± SD of three analyses

	CF	IA	IB
Moisture	8.1 ± 0.1	3.3 ± 0.2	5.5 ± 0.1
Ash	3.7 ± 0.1	2.9 ± 0.3	4.3 ± 0.2
Fibre	18.8 ± 2.0	3.8 ± 0.3	3.2 ± 0.1
Protein content[a]	24.7 ± 1.7	78.0 ± 2.1	88.1 ± 2.7
Lipids	1.5 ± 0.3	3.5 ± 0.4	1.1 ± 0.2
Carbohydrate 'by difference'	51.3 ± 5.5	11.8 ± 1.5	3.3 ± 0.3
Soluble sugars	2.8 ± 0.3	1.3 ± 0.2	0.2 ± 0.01
Dissolved solids	7.2 ± 1.6	12.1 ± 1.9	1.6 ± 0.3
Polyphenols	< 0.1	< 0.1	< 0.1
Nonprotein nitrogen	0.1 ± 0.01	–	–

[a] Total nitrogen × 6.25.

Table 2
Functional properties of chickpea flour (CF) and Isolate-A (IA) and B (IB). Data are the mean ± SD of three analyses

	CF	IA	IB
Solubility[a]	31.8 ± 1.1	26.6 ± 0.9	46.3 ± 3.2
Water absorption[b]	178.8 ± 2.4	343.7 ± 30.1	199.5 ± 4.9
Fat absorption[c]	135.8 ± 6.1	409.4 ± 24.9	125.7 ± 11.2
Emulsion capacity[d]	94.7 ± 0.7	48.1 ± 5.7	76.9 ± 2.2

[a] Percentage of soluble nitrogen in 0.1 M NaCl solution at pH 7.
[b] Grams of water absorbed per 100 g sample.
[c] Grams of fat absorbed per 100 g sample.
[d] Percentage of fat emulsified (% weight).

3.4. Protein composition of chickpea protein isolates

On gel filtration chromatography (Fig. 2a), chickpea flour proteins were fractionated into seven components with molecular masses of 197.2, 101.9, 61.9, 43.6, 18.3, 12.8 and 5.9 kDa (fractions A, B, C, D, E, F and G, respectively). Fraction O is probably made up of protein aggregates because its molecular weight is higher than 3000 kDa. Fraction A was the major fraction and corresponded to the 11S protein of chickpea (Kumar & Venkataraman, 1980). Protein profiles of Isolate-A and B on gel filtration were very different (Fig. 2b and c). Isolate-B showed an enrichment in the 11S protein (fraction A) with respect to chickpea flour. In Isolate-A chromatogram, the 11S protein was not the major fraction but the fractions O and B. The increase of fraction O, respect to chickpea flour, is probably due to a protein polymerization of the proteins during the preparation of the isolate. Molecular weight of fraction B was 61.1 kDa and corresponded to that of the αβ subunits (about 60 KDa) of the 11S proteins. This indicates a partial cleavage of the 11S protein. The different pH values used in the preparation of the isolates is probably the responsible for the differences observed between both gel filtration profiles. The breakdown of 11S type proteins at pH >11 has been demonstrated (Guéguen,

Chevalier, Barbot, & Schaeffer, 1988) and the changes that this dissociation produced (Guéguen, Subriade, Barbot, & Scwenke, 1993) could explain the different functional properties observed for Isolate-A and B. The separation of the 11S protein into its subunits would involve the exposure of the hydrofobic β chains that are hidden in the native legumin (Plietz, Damaschun, & Schwenke, 1980). The accessibility to β chains must facilitate the interaction with non polar compounds like lipids, and thereby the fat absorption should be increased with respect to the whole 11S protein. The dissociation of legumin probably also affected the water absorption since denatured proteins promote the formation of a protein matrices, stabilized by hydrofobic interactions, that are capable of retaining a significant amount of water in their structure (Wagner & Añon, 1990).

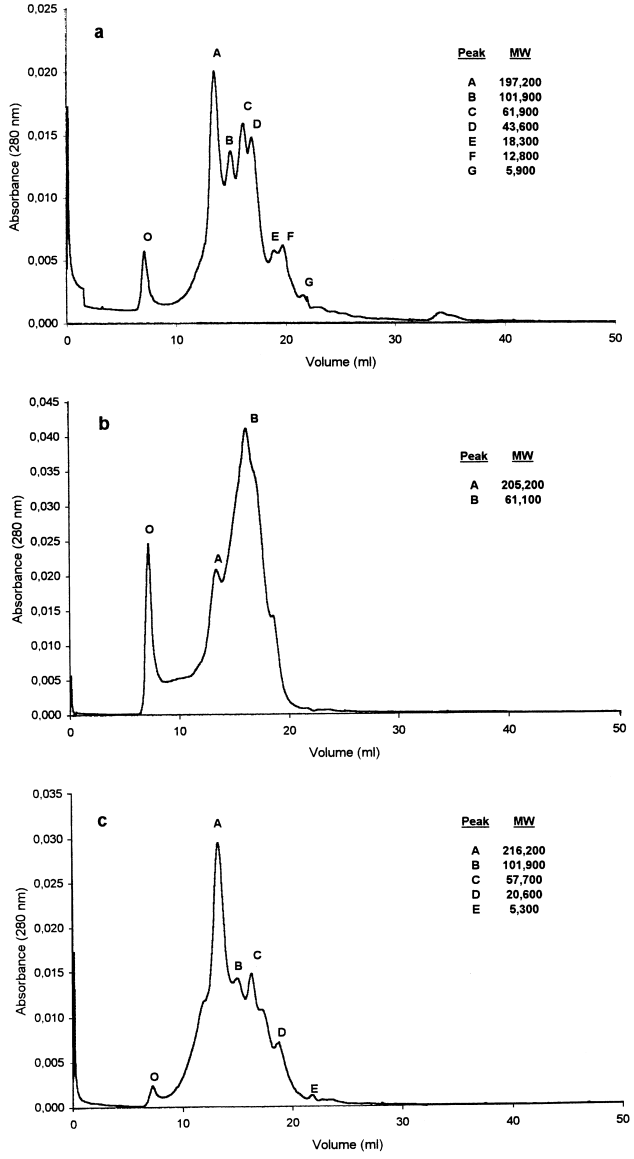

Fig. 2. Gel filtration chromatography of proteins extracted from (a) chickpea flour, (b) Isolate-A and (c) Isolate-B.

SDS-PAGE (Fig. 3) of chickpea proteins showed a complex protein profile characterized by the major presence of proteins in the ranges: 46.5 to 39.8 and 25.3 to 24.3 kDa. These proteins correspond to the polypeptide chains α and β of the 11S protein (Vairinhos & Murray, 1982). Respect to chickpea flour, SDS-PAGE profiles of protein isolates showed an intensification of these bands together with the lightening of three bands in the range of 94, and 27–29 kDa, as a result of the partial elimination of albumins during the preparation of the isolates.

The amino acid composition of the flour and protein isolates from chickpea is shown in Table 3. The flour, and the Isolate-A and B satisfied the FAO requirements (FAO/WHO/UNU, 1985) for the essential amino acids. The chemical scores, based on the content of sulphur amino acids, of the flour and the isolates were above 100. Amino acid analysis indicated a similar composition for both isolates and chickpea flour. The only differences observed were in lysine content (8.5, 7.7 and 7.4 g/16 g N in flour and isolates A and B, respectively) and sulphur amino acid content (3.7, 3.3 and 2.8 g/16 g N in flour, isolate A and isolate B, respectively). The lower content of these amino acids in the isolates, with respect to the flour, are probably due to the high reduction of albumins, which are rich in lysine, cysteine and methionine (Clemente, Sánchez-Vioque, Vioque, Bautista, & Millán, 1998).

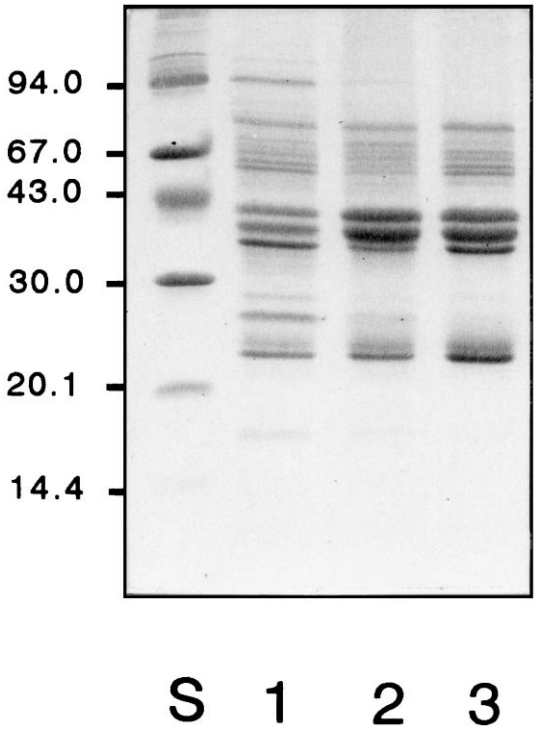

Fig. 3. SDS-PAGE of proteins extracted from chickpea flour (lane 1), Isolate-A (lane 2) and Isolate-B (lane 3). Protein standard kit (lane S) with the molecular masses indicated on the left.

Table 3
Amino acid composition of chickpea flour (CF) and Isolate-A (IA) and B (IB). Data expressed as g 100 g^{-1} protein are the mean ± SD of two analyses

	FAO[a]	CF	IA	IB
Aspartic acid[b]		13.7 ± 0.3	14.2 ± 0.4	13.7 ± 0.3
Glutamic acid[c]		19.5 ± 0.2	19.3 ± 0.2	19.1 ± 0.2
Serine		6.7 ± 0.1	6.8 ± 0.1	7.1 ± 0.1
Histidine	1.9	3.3 ± 0.0	3.2 ± 0.1	3.3 ± 0.0
Glycine		4.8 ± 0.1	4.5 ± 0.0	4.7 ± 0.0
Threonine	3.4	4.7 ± 0.1	4.4 ± 0.1	4.3 ± 0.1
Arginine		12.3 ± 0.1	11.2 ± 0.1	11.8 ± 0.1
Alanine		5.4 ± 0.0	5.2 ± 0.1	5.3 ± 0.1
Tyrosine	6.3[d]	4.0 ± 0.0	4.1 ± 0.1	3.8 ± 0.2
Valine	3.5	5.7 ± 0.1	5.9 ± 0.1	6.0 ± 0.1
Methionine	2.5[e]	2.1 ± 0.1	2.1 ± 0.3	1.6 ± 0.1
Cysteine		1.6 ± 0.2	1.2 ± 0.1	1.2 ± 0.1
Isoleucine	2.8	6.0 ± 0.1	6.2 ± 0.1	6.3 ± 0.0
Leucine	6.6	10.0 ± 0.1	10.4 ± 0.1	10.7 ± 0.1
Phenylalanine		7.9 ± 0.1	8.2 ± 0.1	8.5 ± 0.1
Lysine	5.8	8.5 ± 0.1	7.7 ± 0.1	7.4 ± 0.1

[a] FAO/WHO/UNU Energy and Protein Requirements, 1985.
[b] Aspartic acid + asparagine.
[c] Glutamic acid + glutamine.
[d] Tyrosine + phenylalanine.
[e] Methionine + cysteine.

Protein digestibility of chickpea is mainly affected by the globular structure of the proteins, that hinders the action of digestive enzymes, and by the presence of trypsin and chymotrypsin inhibitors. Since these protease inhibitors are albumins (Richardson, 1991), most of them are removed in the protein isolation procedures, increasing the *in vitro* protein digestibility of Isolate-A (95.6%) and B (96.1%), with respect to the chickpea proteins (76.2%). An approximate 70% reduction in the concentration of trypsin inhibitor has been observed in the process of extraction and precipitation of the isolated soy protein (Waggle et al., 1989). Moreover, chickpea proteins are partially denatured during the preparation of the isolates, being more accessible to digestive enzymes, and improving the hydrolysis (Lynch, Rha, & Catsimpoolas, 1977).

The use of alkaline extraction media, with or without sodium sulfite, and the subsequent precipitation of the proteins at the isoelectric point generates protein isolates principally constituted by the globulin fraction. Although the yields in the preparation of Isolates-A and B are similar, this latter shows a higher protein content as a result of the ethanol and acetone washings. Although the isolates, especially the Isolate B, have a low content of sulphur amino acids, the nutritional quality of the proteins are high due to their high digestibility. The Digestibility corrected Amino acid Score of the Isolate-A and B were 127 and 107, respectively. Chickpea protein isolates show an increase in the content of protein and a higher digestibility with respect to the flour. Because of these improvements, Isolate-A and B are suitable to provide an extra amount of protein in applications such as balanced nutritional foods and high-protein diet products. The colour of an isolate is a serious limitation to the use of proteins in the food industry. Colour of food is darken if the isolate is not light enough. Isolate-B, which colour was practically white, could be very appropriate to lighten the colour and improve the texture of mechanically deboned poultry. Besides the colour, the main differences observed between both isolates are the functional properties that depend on the integrity of the 11S protein. In this sense, the pH used for the protein extraction may be a critical parameter to improve certain physicochemical properties of the isolates. The potential uses of Isolate-A and B as food ingredients are influenced by their different functional properties. Thus, Isolate-A could be suitable for the preparation of cheese or bakery and meat products because of its high water and fat absorption, whereas Isolate-B could be used in foods in which the emulsion capacity is the most important functional property, such as frankfurters or creams.

Acknowledgements

This work was supported by the CSIC ALI95-0734 project. The authors are grateful to Dr. Enrique Martinez-Force for his technical assistance.

References

Alaiz, M., Navarro, J. L., Giron, J., & Vioque, E. (1992). Amino acid analysis by high-performance liquid chromatography after derivatization with diethylethoxymethylenemalonate. *Journal of Chromatography, 591*, 181–186.

AOAC (1990). *Official methods of analysis*, (15th ed.). Arlington: Association of Official Analytical Chemists.

Berot, S., & Davin, A. (1996). Technologie d'extraction et de purification des matières protéiques végétales. In B. Godon (Ed.), *Proteines Végétales*. Paris: Technique & Documentation Lavoisier.

Bhatty, R. S., Sosulski, F. W., & Wu, K. K. (1973). Protein and non-protein nitrogen contents of some oilseeds and peas. *Canadian Journal of Plant Science, 53*(3), 651–657.

Cheftel, J. C., Cuq, J. L., & Lorient, D. (1989). Modificaciones de las proteínas. Modificaciones del valor nutricional y efectos tóxicos, and Propiedades funcionales de las proteínas. In J. F. Cheftel (Ed.), *Proteínas Alimentarias. Bioquímica. Propiedades funcionales. Valor nutritivo. Modificaciones químicas* Chapters 8 and 4. Acribia S.A., Zaragoza.

Chominot, A. (1992). Les Proteagineux dans L'Economie Europeenne des Grandes Cultures. In *Proceedings of the first European Conference on Grain Legumes*. (pp. 529–538). Angers, France.

Clemente, A., Sánchez-Vioque, R., Vioque, J., Bautista, J., & Millán, F. (1998). Effect of cooking on protein quality of chickpea (*Cicer arietinum* L.) seeds. *Food Chemistry*, *62*, 1–6.

De Miguel Gordillo, E. (1991). In *El Garbanzo. Una Alternativa para el Secano*. Madrid: Mundi-Prensa.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*, 350–356.

FAO/WHO/UNU (1985). Energy and protein requirements. Technical report series N° 724, Geneva.

Friedman, M. (1996). Nutritional value of proteins from different food sources. A review. *Journal of Agricultural and Food Chemistry, 44*, 6–29.

Gheyasuddin, S., Cater, C. M., & Mattil, K. F. (1970). Preparation of a colorless sunflower protein isolate. *Food Technology, 24*, 242–243.

Guéguen, J., Chevalier, M., Barbot, J., & Schaeffer, F. (1988). Dissociation and aggregation of pea legumin induced by pH and ionic strength. *Journal of the Science of Food and Agriculture, 44*, 167–182.

Guéguen, J., Subirade, M., Barbot, J., & Schwenke, K. D. (1993). Influence of the dissociation on the surface behaviors of oligomeric seed storage proteins: the example of pea legumin. Act. 4th Symposium. In K. D. Scwenke, R. Mothes, V. C. H. Weinheim (Eds.), *Food proteins structure - functionality relationships*. (pp. 281–289).

Hsu, H. W., Vavak, D. L., Satterlee, L. D., & Miller, G. A. (1977). A multienzyme technique for estimating protein digestibility. *Journal of Food Science, 42*, 1269–1273.

Kikugawa, K., Ido, Y., & Mikami, A. (1981). Studies on peroxidized lipids. VI. Fluorescents products derived from the reaction of primary amines, malonaldehyde and monofunctional aldehydes. *Journal of the American Oil Chemists' Society, 61*, 1574–1581.

Kumar, K. G., & Venkataraman, L. V. (1980). Chickpea seed proteins: Isolation and characterization of 10.3 S protein. *Journal of Agricultural and Food Chemistry, 28*(3), 524–529.

Laemmli, U. K. (1970). Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature, 227*, 680–685.

Lee, S. C., Prosky, L., & De Vries, J. W. (1992). Determination of total, soluble and insoluble dietary fiber in foods-enzymatic-gravimetric method, MES-TRIS buffer: Collaborative study. *Journal of AOAC International, 75*(3), 395–416.

Lin, M. J. Y., Humbert, E. S., & Sosulski, F. W. (1974). Certain functional properties of sunflower meal products. *Journal of Food Science, 39*, 368–370.

Lynch, C. J., Rha, C. K., & Catsimpoolas, N. (1977). Tryptic hydrolysis of glycinin and its subunits. *Journal of the Science of Food and Agriculture, 28*, 971–979.

Moores, R. G., Mc Dermott, D. L., & Wood, T. R. (1948). Determination of chlorogenic acid in coffee. *Analytical Chemistry, 20*, 620–624.

Morr, C. V., German, B., Kinsella, J. E., Regenstein, J. M., Van Buren, J. P., Kilara, A., Lewis, B. A., & Mangino, M. E. (1985). A collaborative study to develop a standardized food protein solubility procedure. *Journal of Food Science, 50*, 1715–1718.

Nash, A. M., Eldridge, A. C., & Wolf, W. J. (1967). Fractionation and characterization of alcohol extractables associated with soybean proteins. Nonprotein components. *Journal of Agricultural and Food Chemistry, 15*, 102–108.

Plietz, P., Damaschun, G., & Schwenke, K. D. (1980). Quaternary structure of 11S globulin from different plant seeds. *Studia Biophysica, 79*, 145–146.

Pomenta, J. V., & Burns, E. E. (1971). Factors affecting chlorogenic, quinic and caffeic acid in sunflower kernels. *Journal of Food Science, 36*, 490–492.

Rackis, J. J., Sessa, D. J., & Honing, D. H. (1979). Flavor problems of vegetable food proteins. *Journal of the American Oil Chemists' Society, 56*, 262–271.

Richardson, M. (1991). Seed storage proteins: The enzyme inhibitors. In L. J. Rogers (Ed.), *Methods in Plant Biochemistry*, (Vol 5). Academic Press Limited, London.

Sánchez-Vioque, R., Clemente, A., Vioque, J., Bautista, J., & Millán, F. (1998a). Neutral lipids of chickpea flour and protein isolates. *Journal of the American Oil Chemists' Society*, *75*, 851–855.

Sánchez-Vioque, R., Clemente, A., Vioque, J., Bautista, J. & Millán, F. (1998b). Polar lipids of defatted chickpea (*Cicer arietinum* L.) flour and protein isolates. *Food Chemistry*, *63*, 357–361.

Singh, H., & Privett, O. S. (1970). Studies on the glycolipids and phospholipids of immature soybeans. *Lipids, 5*, 692–697.

Singh, U. (1988). Antinutritional factors of chickpea and pigeonpea and their removal by processing. *Plant Foods for Human Nutrition, 38*, 251–261.

Sosulski, F. W. (1962). The centrifuge method for determining flour absorption in hard red spring wheats. *Cereal Chemistry, 39*, 344–350.

Ulloa, J. A., Valencia, M. E., & Garcia, Z. H. (1988). Protein concentrate from chickpea: Nutritive value of a protein concentrate from chickpea (*Cicer arietinum*) obtained by ultrafiltration and its potential use in an infant formula. *Journal of Food Science, 53*(5), 1396–98.

Vairinhos, F., & Murray, D. R. (1982). Changes in polypeptide composition during seed development in chickpea, *Cicer arietinum* L.. *Z. Pflanzenphysiol. Bd., 106*, 447–452.

Waggle, D. H., Steinke, F. H., & Shen, J. L. (1989). Isolated soy proteins. In R.H. Matthews (Ed.), *Legumes. Chemistry, Technology and Human Nutrition*. New York: Marcel Dekker.

Wagner, J. R., & Añon, M. C. (1990). Influence of denaturation, hydrofobicity and sulfhydryl content on solubility and water absorption capacity of soy protein isolates. *Journal of Food Science, 55*, 765–770.