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Polar lipids of defatted chickpea (*Cicer arietinum* L.) flour and protein isolates

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Polar lipid composition of defatted chickpea flour and protein isolates was studied. The main compounds were phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine. Other compounds, in lower amounts, were sterol glucosides, esterified sterol glucosides and digalactosyldiglycerides. Palmitic, oleic and linoleic acid were the main fatty acids in polar lipids. A reduction in the content of phosphatidylinositol and sterol glucosides in the protein isolates with respect to the defatted flour was observed, indicating that these compounds are more sensitive to the chemical treatment of the protein isolates. However, unsaturated fatty acids and unsaturated sterols content decreased in the protein isolates probably undergoing oxidative degradation. © 1998 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

Plant proteins are an alternative to proteins from animal sources in human nutrition. Among plants, legumes are an important source of proteins, carbohydrate, minerals and vitamins. Legume seeds contain two or three times more proteins than cereals. Their high proportion of lysine, adequate amounts of carbohydrates and low lipid content, make them a suitable source to complement cereals in human nutrition.

Among legumes, the chickpea (*Cicer arietinum* L.) is the third most important grain legume in the world on the basis of total grain production (FAO, 1992). It is broadly cultivated in temperate areas of different parts of the world, mainly in India, South America and the Mediterranean region.

Plant protein isolates have been the object of increasing interest over recent years. Industrial production of plant protein isolates is cheaper than production from animal sources and the risks of transmission of animal diseases are avoided. Chickpea is considered a good starting material in the food industry because of its protein quality, which is comparable with that of the soybean (Singh, 1985; Friedman, 1996), and the fact that it could be processed to obtain protein isolates (Adler-Nissen, 1986).

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The presence of lipids in protein isolates, even in small amounts, is important because of the influence of lipids on the nutritional quality, acceptability and functional characteristics of the isolates. Interactions of proteins with lipids may produce changes in the conformation pattern of the protein, modifying and affecting the characteristics of the protein isolates. Also, unsaturated fatty acids, abundant in chickpea lipids, could be oxidized producing chemical compounds that could react with amino acid side chains, decreasing the biological value and quality of the product (Kikugawa *et al.*, 1981; Rackis *et al.*, 1979). Thus, knowledge of the nature and quantity of the lipids associated with the isolates could be of interest in preventing these changes and deteriorations in the final product.

The study of polar lipids associated with protein isolates is not very extended, although it has been studied in some crops such as sunflower or lupins (Millan *et al.*, 1983, 1984, 1994). Several studies on the total lipid composition of chickpea seeds have been reported (Vioque and Maza, 1970; Ghirardi *et al.*, 1974; Sosulski and Gadan, 1988) but no data on lipid composition in chickpea protein isolates are available in the literature.

The objective of this paper was to resolve, identify and quantify the polar lipids of defatted chickpea flour and protein isolates in order to know how these compounds may affect the fundamental characteristics of

the proteins such as digestibility, amino acid bioavailability and functional properties.

MATERIAL AND METHODS

Plant material

Chickpea seeds (c.v. Athenas), cultivated in the experimental fields of Carmona (Sevilla), were a gift from Koipesol Semillas, S.A. Seeds were cleaned and freed from broken seeds, dust and other foreign materials. Chickpea flour was prepared by milling the seeds at room temperature with a domestic grinder.

Determination of moisture, ash, nitrogen, carbohydrates and fiber

Moisture, ash and nitrogen content were determined according to AOAC approved methods (AOAC, 1990). Total fiber was determined according to the procedure described by Lee *et al.* (1992). Total carbohydrates were calculated as the difference referred to 100% with moisture, ash, fiber, protein and lipid content.

Preparation of protein isolates

Chickpea flour was defatted for 9 h using boiling hexane in a soxhlet system. Two types of protein isolates were prepared from defatted chickpea flour. Isolate A was obtained by dispersing defatted chickpea flour in 0.2% NaOH solution at pH 12 at a ratio 1:10 (w:v) and extracted by shaking for 1 h. After centrifugation at $8000 \times g$ for 15 min, two additional extractions were carried out using half of the volume of alkaline solution. Supernatants were pooled and the pH adjusted to 4.3, which corresponds to the isoelectric point of chickpea proteins. After centrifugation at $8000 \times g$ for 15 min, the precipitate was washed with distilled water adjusted at pH 4.3, frozen at −20°C and lyophilized. Isolate B was prepared as above but using a 0.25% Na_2SO_3 solution at pH 10.5 for the extraction of proteins. Supernatants were pooled and the pH adjusted to the isoelectric point of chickpea proteins as for isolate A. The precipitate was successively washed with distilled water (pH 4.3), 96% ethanol and acetone, and dried at room temperature.

Lipid extraction

Lipids in the defatted chickpea flour and protein isolates were extracted using 86% ethanol in a proportion 1:20 (w:v) for 36 h at room temperature (Nash *et al.*, 1967). The ethanol solution was transferred to a separatory funnel and a mixture of 1:1 chloroform and 0.5% NaCl aqueous solution was added. The chloroform phase was extracted according to Singh and Privett (1970) to remove nonlipid compounds.

Fractionation of lipids

A preliminary separation of lipids was performed by the method of Singh and Privett (1970) using an acid-treated Florisil (Merck, Germany) column. The fractionation of the different lipid components was carried out using chloroform, chloroform: acetone (1:1) and methanol to elute neutral lipids, glycolipids and phospholipids, respectively. The elution was monitored by thin-layer chromatography (TLC) with silica gel 60G (Merck, Germany) 0.25 mm layer plates. Definitive separation and isolation of polar lipids was achieved by TLC using the solvent systems chloroform:methanol:7 N ammonia (90:25:2) for sterol glucosides and digalactosyldiglycerides; chloroform:methanol:7 N ammonia (100:15:2) for esterified sterol glucosides and chloroform: methanol:acetic acid:water (85:15:10:3) for phospholipids. Lipids were visualized with iodine vapors.

Quantification of lipid components

Quantification of phospholipids, esterified sterol glucosides and digalactosyldiglycerides was performed using the hydroxamic method (Vioque and Holman, 1962). Fatty acid esters react with hydroxylamine and Fe^{3+} to produce fatty acid derivatives with an absorption peak at 520 nm. A calibration curve of myristic acid methyl ester was used.

Quantification of sterol glucosides was carried out according to Huang *et al.* (1961) based on the colorimetric reaction (absorption peak at 550 nm) between the sterols and a chemical reagent constituted by sulfuric acid: acetic acid: acetic anhydride (1:3:6) and 2% sodium sulfate. A calibration curve of β-sitosterol was used.

Gas liquid chromatography (GLC)

A Hewlett-Packard, GC 5890 model series II, fitted with a flame ionization detector and a HP 3390A integrator (Palo Alto, CA) was used. Hydrogen at 12 psi column head pressure and 1 ml/min flow rate was employed as the carrier gas. Nitrogen was used as an auxiliary gas. Fatty acids were determined as methyl derivatives using a Hewlett Packard-20M 25 m × 0.2 mm × 0.2 μm capillary column. Fatty acid derivation was carried out using 2.5% H_2SO_4 in anhydrous methanol: benzene (9:1). Injector, detector and oven temperatures were maintained at 225, 250 and 170°C, respectively. Sterols were derivated using a mixture of pyridine: hexamethyldisilane: trimethylchlorosilane (9:3:1) at room temperature. GLC analyses were performed using a TRB-1 30 m × 0.25 mm × 0.25 μm capillary column (Supelco, PA). Injector and detector temperatures were maintained at 300°C. The oven temperature was maintained at 265°C for sterol analyses.

RESULTS AND DISCUSSION

The polar lipid composition of defatted chickpea flour and two types of protein isolates were determined. Flour was defatted previously with hexane in a soxhlet apparatus. In spite of the defatting process, the lipids were not removed completely, ranging from 1% in isolate B to 3.4% in isolate A (Table 1). The washes with ethanol and acetone contributed to the reduction in the lipid content in the protein isolate B.

Lipids can be classified as glycolipids, phospholipids and neutral lipids according to the fractionation method of Singh and Privett (1970). Polar lipids, constituted by glycolipids and phospholipids, are the main fraction of the lipids remaining in the flour and protein isolates, representing between 86.6% (flour) and 88.4% (isolate A) (Table 2).

The main polar lipids observed are phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine, with minor amounts of sterol glucosides, esterified sterol glucosides and digalactosyldiglycerides (Table 3). Thus, phospholipids predominate in polar lipids of defatted chickpea flour and protein isolates ranging in content from 96.5% in the flour to 98.8% in isolate B. This predominance of phospholipids can be explained by the abundance, in the cells of mature seeds, of organelles such as endoplasmic reticulum or Golgi apparatus that have a high proportion of phospholipids in their membranes. However, chloroplasts, where glycolipids predominate, are almost absent in the dry seeds.

Differences in phospholipid composition between the protein isolates and the defatted flour were observed (Table 3). The phosphatidylethanolamine content is clearly increased in the protein isolates with respect to the flour. On the contrary, phosphatidylinositol average values are lower in the protein isolates while amounts of phosphatidylcholine are similar. These results suggest that phosphatidylinositol is affected more by the obtention process of protein isolates. With respect to glycolipids, the content of sterol glucosides also decreases in the protein isolates, more clearly in isolate B. This result is probably because the glucosides are removed efficiently by the ethanol and acetone washes.

The fatty acid composition of polar lipids was studied (Table 4). The main fatty acids are $C_{16:0}$, $C_{18:1}$ and $C_{18:2}$. In the fatty acid composition of membrane lipids, such as phospholipids and glycolipids, polyunsaturated fatty acids predominate, mainly $C_{18:2}$ or $C_{18:3}$ (Joyard *et al.*, 1991). However, the content of $C_{18:3}$ in polar lipids of defatted chickpea flour and protein isolates is lower, probably because it is oxidized during the milling of the seeds (Kates, 1986). In protein isolates, a decrease in unsaturated fatty acids from polar lipids with respect to the flour is generally observed. This fact could be due to degradation by auto-oxidation during the process of isolation. Nevertheless, there is no clear relationship between the oxidation of the unsaturated fatty acids and the amounts of the different polar lipid classes obtained (Table 3). The decrease in the content of unsaturated fatty acids of phosphatidylinositol in isolate A with respect to the chickpea flour (1.8%), is not large enough to explain the reduction in the amounts of this lipid in the protein isolate. This may indicate that the decrease in some polar lipids in the isolates is not only due to the oxidation but also to their different capacity to bind to the proteins.

The sterol composition of polar lipids in the flour and protein isolates has been determined (Table 5). Sterols are common components of cellular membranes, the main ones in plants being β-sitosterol, campesterol and stigmasterol (Benveniste, 1986). These compounds are also the most abundant in sterol glucosides and esterified sterol glucosides of chickpea (Table 5), β-sitosterol representing more than 80% of the glycolipids in defatted chickpea flour and protein isolates. A reduction in the content of stigmasterol and δ-5-avenasterol from protein isolates with respect to the defatted flour was observed. These

Table 1. Chemical composition (%) of defatted chickpea flour and protein isolates[a]

Constituents	DCF[b]	Isolate A[c]	Isolate B[d]
Moisture	8.1 ± 0.1	3.3 ± 0.2	5.5 ± 0.1
Ash	3.4 ± 0.1	2.8 ± 0.3	4.1 ± 0.2
Protein[e]	22.7 ± 1.7	75.0 ± 2.5	83.3 ± 0.9
Fiber	17.3 ± 2.0	3.7 ± 0.3	3.0 ± 0.1
Lipid	1.4 ± 0.3	3.4 ± 0.4	1.0 ± 0.2
Carbohydrates	47.1 ± 10.5	11.8 ± 1.5	3.1 ± 0.3

[a]Data are the mean ± SD of three determinations.
[b]Defatted chickpea flour.
[c]Chickpea protein isolate obtained with NaOH.
[d]Chickpea protein isolate obtained with Na_2SO_3.
[e]Nitrogen × 6.25.

Table 2. Lipid composition (%) of defatted chickpea flour and protein isolates[a]

	DCF[b]	Isolate A[c]	Isolate B[d]
Neutral lipids	13.4 ± 2.2	11.6 ± 1.6	11.9 ± 0.3
Polar lipids	86.6 ± 2.2	88.4 ± 1.6	88.1 ± 0.3

[a]Data are the mean ± SD of three determinations.
[b]Defatted chickpea flour.
[c]Chickpea protein isolate obtained with NaOH.
[d]Chickpea protein isolate obtained with Na_2SO_3.

Table 3. Polar lipid composition (%) of defatted chickpea flour and protein isolates[a]

	DCF[b]	Isolate A[c]	Isolate B[d]
Phosphatidylethanolamine	7.6 ± 2.7	19.0 ± 2.6	32.8 ± 1.8
Phosphatidylcholine	62.5 ± 4.1	72.4 ± 1.4	57.4 ± 1.3
Phosphatidylinositol	26.4 ± 1.3	6.8 ± 1.2	8.6 ± 3.0
Esterified sterol glucoside	traces	traces	traces
Sterol glucoside	3.5 ± 0.6	1.8 ± 0.7	1.2 ± 0.2
Digalactosildiglyceride	traces	traces	traces

[a]Data are the mean ± SD of three determinations.
[b]Defatted chickpea flour.
[c]Chickpea protein isolate obtained with NaOH.
[d]Chickpea protein isolate obtained with Na_2SO_3.

Table 4. Fatty acid composition (%) of polar lipids of defatted chickpea flour and protein isolates[a]

	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Phosphatidylethanolamine						
DCF[b]	traces	17.2±0.1	2.6±0.1	28.7±0.1	50.3±0.1	1.2±0.1
Isolate A[c]	1.5±0.1	16.0±0.1	3.0±0.5	28.0±0.1	50.2±0.3	1.3±0.1
Isolate B[d]	traces	20.7±0.1	2.6±0.1	31.9±0.1	44.8±0.1	traces
Phosphatidylcholine						
DCF	traces	14.7±0.5	2.2±0.1	31.4±0.4	50.4±0.8	1.3±0.1
Isolate A	1.6±0.1	24.1±0.1	4.6±0.1	24.8±0.3	43.8±0.1	1.1±0.1
Isolate B	traces	15.1±0.1	2.7±0.1	34.6±0.1	46.6±0.3	1.0±0.1
Phosphatidylinositol						
DCF	traces	41.6±0.5	2.1±0.1	13.3±0.1	41.5±0.4	1.5±0.1
Isolate A	3.5±0.6	38.0±0.3	4.0±1.1	16.0±0.3	37.0±0.5	1.5±0.1
Isolate B	traces	45.4±0.2	6.4±0.1	12.8±0.6	35.4±0.3	traces
Esterified sterol glucoside						
DCF	traces	35.9±1.8	13.2±0.4	17.3±0.4	33.6±0.8	traces
Isolate A	traces	33.8±0.1	9.3±0.7	20.3±0.7	36.6±1.3	traces
Isolate B	traces	61.0±1.1	14.2±0.3	15.0±0.1	9.8±1.2	traces
Digalactosildiglyceride						
DCF	traces	36.9±0.1	3.8±0.1	14.7±0.1	42.5±0.1	2.1±0.1
Isolate A	traces	36.9±1.1	3.4±0.1	13.1±0.3	44.0±0.6	2.6±0.1
Isolate B	traces	36.7±0.4	3.6±0.1	14.6±0.1	43.1±0.3	2.0±0.1

[a]Data are the mean ± SD of three determinations.
[b]Defatted chickpea flour.
[c]Chickpea protein isolate obtained with NaOH.
[d]Chickpea protein isolate obtained with Na_2SO_3.

Table 5. Sterol composition (%) of polar lipids of defatted chickpea flour and protein isolates[a]

	Campesterol	Stigmasterol	β-Sitosterol	δ-5-Avenasterol
Sterol glucoside				
DCF[b]	10.0±0.1	5.4±0.1	83.4±0.1	1.2±0.1
Isolate A[c]	10.3±0.6	5.6±0.1	83.1±0.6	1.0±0.1
Isolate B[d]	8.4±0.1	4.7±0.1	86.1±0.1	0.8±0.1
Esterified sterol glucoside				
DCF	8.5±0.2	6.0±0.4	83.3±0.2	2.2±0.1
Isolate A	9.3±0.1	5.0±0.1	85.7±0.2	traces
Isolate B	8.6±0.7	5.6±0.5	83.8±1.1	2.0±0.1

[a]Data are the mean ± SD of three determinations.
[b]Defatted chickpea flour.
[c]Chickpea protein isolate obtained with NaOH.
[d]Chickpea protein isolate obtained with Na_2SO_3.

compounds seem to be more sensitive to obtain protein isolates, probably because of the higher reactivity of the side chain containing one unsaturation which is absent in β-sitosterol and campesterol.

The polar lipids found in chickpea flour and protein isolates constitute a group of compounds with different chemical characteristics. These lipids can interact with proteins and influence the functional characteristics and quality of the product. The presence of polyunsaturated fatty acids, such as $C_{18:2}$, may increase lipid oxidation and peroxidation, inducing the polymerization of proteins and consequently a decrease in the protein solubility.

Polar lipids associated with defatted chickpea flour and protein isolates are characterized by the abundance of phospholipids, mainly phosphatidylcholine.

A reduction in the content of phosphatidylinositol and sterol glucosides during the obtention of protein isolates was observed and was related to the degradative processes of these compounds. A decrease in unsaturated sterols and fatty acids from the polar lipids of protein isolates is observed.

We have obtained a greater knowledge of the nature and quantity of the polar lipids in the chickpea protein isolates. This information will be useful for the overall comprehension of the interactions between lipids and proteins, and how these interactions can be prevented or modified in order to maintain, or even improve, the quality of protein isolates.

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