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Interaction of Chickpea (*Cicer arietinum* L.) Legumin with Oxidized Linoleic Acid

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Chickpea legumin has been purified and incubated under oxidizing conditions with linoleic acid to investigate the influence of this acid on the structure and nutritional quality of the protein. At the end of the incubation time, >30% of the linoleic acid was oxidized. The oxidized linoleic acid was highly detrimental to legumin, and the electrophoretic pattern of the protein was completely changed after the incubation period. Nevertheless, neither polymerization nor cleavage of the protein was observed as deduced from gel filtration chromatography, suggesting that the changes observed in native electrophoresis were probably due to oxidation of legumin. The incubation of legumin with linoleic acid also produced a diminution of the contents of methionine and histidine, by 81.3 and 24.3%, respectively. Finally, in vitro protein digestibility of chickpea legumin was also seriously affected by the incubation with linoleic acid, decreasing from 84.1 to 69.2%.

Keywords: *Chickpea; Cicer arietinum L.; legumin; linoleic acid; oxidized lipid−protein interaction*

INTRODUCTION

Legumin is the main storage protein of chickpea seeds. It is made up of six $\alpha\beta$ subunits organized in a trigonal antiprism by noncovalent bonds. Each α chain is linked to a β chain by disulfide bonds. The hydrophilic α chains are in the outer part of the molecule, whereas the β chains constitute the hydrophobic heart of the protein (Plietz et al., 1980). The approximate molecular mass is 360 kDa. During the preparation of chickpea protein isolates, proteins are precipitated at the isoelectric point, legumin being the major component of the final product (Sánchez-Vioque et al., 1999). Although protein isolates contain >90% protein, other compounds are present in the isolates, such as minerals, polyphenols, lipids, and sugars (Waggle et al., 1989). Among these components, lipids are one of the most important because of their influences on the functional properties, nutritional quality, and acceptability of the product. In chickpea protein isolates lipid contents range between 1 and 3.4% (Sánchez-Vioque et al., 1998a). Association of lipids with proteins occurs during the preparation of the isolate and is favored by the denaturation of the proteins during the process. This denaturation results in exposure of the hydrophobic amino acids, hidden in the native protein, and the subsequent association between the aliphatic chains of lipids and these amino acids by hydrophobic unions (Hanssens et al., 1985). Other types of lipid−protein interactions are the electrostatics bonds (Keenan et al., 1982), hydrogen bonds (Gardner, 1979), and even metal bridges (Fullington, 1969). These interactions play an important role in the functional properties of the proteins and even affect certain technological processes, such as protein extrusion (Arêas, 1986). Thus, lipids decrease the solubility of the protein isolates (Boatright and Hettiarachchy, 1995), and the fatty acid composition of the lipids influences the emulsifying properties of the proteins (Karleskind et al., 1996). Besides, lipids associated with protein isolates are susceptible to oxidation during the processing and storage of the product. Lipid oxidation leads to formation of many compounds such as hydroperoxides and their secondary degradation products (aldehydes, ketones, hydrocarbons, etc.) (Hamilton et al., 1997). These compounds are responsible for unacceptable flavors and cause many changes in the functional and nutritional properties of the isolates as a result of their covalent interaction with proteins. Several amino acids, mainly cysteine, methionine, histidine, tryptophan, tyrosine, and lysine, are affected by the oxidation products of lipids, such that bioavailability is reduced (Hidalgo et al., 1992).

Most studies on lipid−protein interactions have been done in proteins from animal sources, such as milk (Nielsen et al., 1985; Hidalgo and Kinsella, 1989), fish (Braddock and Dugan, 1973), or egg (Pokorny et al., 1988). Despite the interest in lipid−protein interactions, there are few studies related to the modification of vegetable protein by oxidized lipids (Damodaran and

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Kinsella, 1981a,b). To our knowledge, this is the first report on chickpea protein interactions with lipids. Chickpea protein isolates are especially sensitive to lipid oxidation because of the unsaturated nature of their lipids (Sosulski and Gadan, 1988). Linoleic acid, the major fatty acid present in chickpea protein isolates (Sánchez-Vioque et al., 1998a,b), has been used to investigate its interaction with legumin. Results obtained have implications on the storage and processing of chickpea protein isolates. This work deals with the deteriorative lipid changes on legumin and the importance of these alterations on the nutritional value of chickpea protein isolates.

MATERIALS AND METHODS

Materials. Chickpea seeds were a gift from Koipesol Semillas, S.A. (Sevilla, Spain). Trypsin (EC 3.4.21.4), α-chymotrypsin (EC 3.4.21.1), peptidase, tris(hydroxymethyl)aminomethane (Tris), bromophenol blue, ethylendiaminetetraacetic acid, disodium salt (EDTA), dithiothreitol (DTT), sodium dodecyl sulfate (SDS), 2,4,6-trinitrobenzenesulfonic acid (TNBS), D,L-α-aminobutyric acid, L-lysine monohydrochloride, and linoleic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Triton X-100 was obtained from Rohm & Haas Co. (Philadelphia, PA). Acrylamide, *N*,*N*′-methylenebis(acrylamide), and Coomassie Brilliant Blue G-250 were purchased from Serva (Heidelberg, Germany). Standards for electrophoresis and gel filtration, Sephacryl S-300, Superose 6 H 10/30 column, and PD-10 Sephadex G-25M columns were supplied by Pharmacia Biotechnology (Uppsala, Sweden). Protein Pak DEAE 8 H 1000 Å 8 μm was purchased from Waters (Milford, MA) and diethyl ethoxymethylenemalonate from Fluka (Buchs, Switzerland). All other chemicals were of analytical grade.

Purification of Legumin. Chickpea seeds (100 g) were ground with a domestic blender (particle size, 0.2−0.5 mm) and extracted under stirring with 1000 mL of 0.1 M sodium borate buffer (pH 8.3) at room temperature for 1 h, according to the method of Singh et al. (1988). The extract was centrifuged at 8000*g* for 15 min. The supernatant was dialyzed against 25 mM sodium citrate buffer (pH 4.6) at 4 °C for 15 h and centrifuged as before. The supernatant and pellet were the albumin and globulin fractions, respectively. Globulin precipitate was dissolved in 0.1 M sodium borate and 0.2 M sodium chloride buffer (pH 8.3), dialyzed against 25 mM sodium citrate buffer (pH 4.6) at 4 °C for 15 h, and centrifuged. The supernatant was discarded, and the precipitated legumin fraction was freeze-dried. The legumin fraction was dispersed by shaking in 0.1 M sodium borate and 0.2 M sodium chloride buffer (pH 8.3) (1:20 w/v), and insoluble materials were eliminated by centrifugation at 8000*g* for 15 min. The supernatant was eluted with 0.19 M sodium phosphate and 19 mM sodium citrate buffer (pH 7.0) in a PD-10 Sephadex G-25M column to remove non-protein compounds. The legumin solution was then loaded in a Protein Pak DEAE 8 H 1000 Å 8 μm ion exchange column. Protein purification was carried out using a binary gradient system. The solvents used were as follows: buffer A, 0.19 M sodium phosphate, 19 mM sodium citrate buffer (pH 7.0); buffer B, 0.19 M sodium phosphate, 19 mM sodium citrate, 0.5 M sodium chloride buffer (pH 7.0). Solvents were delivered to the column as follows: time 0−25 min, elution with 100% buffer A; 25−35 min, linear gradient from 0 to 20% buffer B; 35−45 min, elution with 20% buffer B; 45−55 min, linear gradient from 20 to 50% buffer B; 55−60 min, elution with 50% buffer B; 60−65 min, linear gradient from 50 to 100% buffer B; 65−70 min, elution with 100% buffer B. Flow rate was 1 mL/min, and volume injection and sample concentration were 10 mL and 18 mg of protein/mL, respectively. Eluent from the column was monitored by SDS−PAGE, and legumin-enriched fractions were pooled for further purification by gel filtration chromatography. Purification of legumin by gel filtration was performed in a Sephacryl S-300 74 × 2.6 cm column. Samples were eluted with 0.1 M sodium borate and 0.2 M sodium chloride buffer (pH 8.3). The flow rate was 0.7 mL/min. Volume injection and sample concentration were 5 mL and 20−30 mg of protein/mL. Elution of the column was monitored by SDS−PAGE as above, and legumin-enriched fractions were pooled. After gel filtration, the purity of the legumin was considered suitable for lipid−protein studies as monitored by native PAGE.

Incubation of Legumin with Linoleic Acid. Legumin (2 mg/mL) dissolved in 0.2 M sodium borate an 0.03% Triton X-100 buffer (pH 9.0) was mixed by sonication with linoleic acid (ratio 1:2 w/w), bubbled with air for 15 min, and incubated at 37 °C for 48 h (Millán, 1983). After incubation, the sample was extracted three times with hexane to recover the linoleic acid. A control incubation was prepared as described above but without the addition of linoleic acid and hence without its extraction with hexane.

Linoleic Acid Oxidation. Linoleic acid was quantitated by gas−liquid chromatography using stearic acid as an internal standard, and a decrease in linoleic acid content was attributed to lipid oxidation (Chen and Nawar, 1991). For gas−liquid chromatography, a Hewlett-Packard GC 5890 model series II, fitted with a flame ionization detector and an HP 3390A integrator (Palo Alto, CA), was used. Hydrogen at 12 psi column head pressure and 1 mL/min flow was employed as a carrier gas. Nitrogen was used as an auxiliary gas. Linoleic acid was derivatized with 2.5% H_2SO_4 in anhydrous methanol/benzene (9:1) at 75 °C for 2 h. Samples were injected in a Hewlett-Packard 20 M 25 m × 0.2 mm × 0.2 μm capillary column. Injector, detector, and oven temperatures were maintained at 225, 250, and 170 °C.

Electrophoresis. Native polyacrylamide gel electrophoresis (native PAGE) and sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) were performed using 0.75 mm thick gels and run at 20 mA in a Mini Protean unit (Bio-Rad, Richmond, CA). Gels were stained with 0.25% Coomassie Brilliant Blue G-250 in water/methanol/acetic acid (5:5:1) for 2 h and destained first with water/methanol/acetic acid (13:5:2) for 1 h and finally with 5% acetic acid.

Native PAGE. Continuous gels were prepared as follows: 4% (w/v) acrylamide/bis(acrylamide) (97.3:2.7), 62 mM Tris (pH 6.8) for the stacking gel; and 8% (w/v) acrylamide/bis(acrylamide) (97.3:2.7), 375 mM Tris (pH 8.8) for the separating gel. The running buffer was 25 mM Tris and 190 mM glycine (pH 8.4). Protein samples (2 mg/mL) were mixed 1:1 (v/v) with a solubilization buffer containing 62 mM Tris, 10% glycerol, and 0.01% bromophenol blue (pH 6.8).

SDS−PAGE. Gels were prepared as follows: 3% (w/v) acrylamide/bis(acrylamide) (99:1), 125 mM Tris, and 0.1% SDS (pH 6.8) for the stacking gel; and 20% (w/v) acrylamide/bis(acrylamide) (99:1), 375 mM Tris, and 0.1% SDS (pH 8.8) for the separating gel. The running buffer was the same as used for native PAGE but with 0.1% SDS (Laemmli, 1970). Protein samples (2 mg/mL) were mixed 1:1 (v/v) with a solubilization buffer of 80 mM Tris, 0.57% EDTA, 0.26% DTT, 20% sucrose, 3.3% SDS, and 0.008% bromophenol blue (pH 6.8) and reduced with 2-mercaptoethanol in boiling water. The molecular weights of proteins were determined using the following standards: phosphorylase *b* (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa).

Gel Filtration Liquid Chromatography of the Products from the Incubation of Legumin with Linoleic Acid. Samples (2 mg/mL) were loaded on a Superose 6 H 10/30 column and eluted with 50 mL of 0.1 M sodium borate and 0.2 M sodium chloride buffer (pH 8.3). Volume injection and flow rate were 200 μL and 0.4 mL/min, respectively. Elution of the proteins was monitored using Bradford's reagent (Bradford, 1976). The molecular masses were determined using Blue Dextran 2000 (2000 kDa), thyroglobulin (669 kDa), β-amylase (200 kDa), bovine serum albumin (67 kDa), and ribonuclease (13.7 kDa) as molecular mass standards.

Amino Acid Analysis. Utilization of routine methods of high-performance liquid chromatography (HPLC) for the analysis of altered amino acids has the drawback that modified

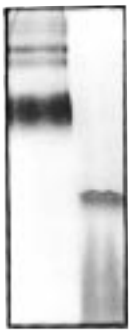

Figure 1. Native PAGE of the control (C) and legumin plus linoleic acid (48 h) after incubation at pH 9.0 and 37 °C for 48 h.

structures can revert to the original amino acids during the acid hydrolysis of the protein, making impossible the quantitation of amino acid losses. To overcome this limitation, it has been proposed that a preliminary reduction of the proteins be included to make the modified structures of amino acids resistant to the acid hydrolysis (Alaiz and Girón, 1994; Lewish and Levine, 1995). Samples were reduced with $NaBH_4$ at 37 °C for 15 min (Alaiz and Girón, 1994), and the acid hydrolysis of the proteins was performed with 6 N HCl at 110 °C for 24 h. After hydrolysis, samples were dried and dissolved in 1 M sodium borate buffer (pH 9.0). Amino acids were derivatized at 50 °C for 50 min with 0.8 μL of diethyl ethoxymethylenemalonate/mL of solution and determined by reversed-phase HPLC according to the method of Alaiz et al. (1992). D,L-α-Aminobutyric acid was used as an internal standard. The HPLC apparatus (Waters) consisted of a model 600E multisolvent 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 reversed phase column (Nova-Pak C_{18}, 4 μm, Waters) using a binary gradient system with (A) 25 mM sodium acetate (pH 6.0) and (B) acetonitrile. Solvents were delivered to the column as follows: time 0−3 min, linear gradient from A/B (91:9) to A/B (86:14); 3−13 min, elution with A/B (86:14); 13−30 min, linear gradient from A/B (86:14) to A/B (69:31); 30−35 min, elution with A/B (69:31). The column was maintained at 18 °C by a temperature controller.

Determination of methionine losses by acid hydrolysis of the protein is useless because the main oxidation products derived from this amino acid are converted to methionine under acidic conditions (Ray and Koshland, 1960). Methionine content was quantitated without reduction and after hydrolysis with 5 N KOH at 110 °C for 16 h (Schachter et al., 1963). After hydrolysis, samples were adjusted to pH 9, derivatized, and resolved as described above.

Available Lysine. Available lysine was determined according to the method of Kakade and Liener (1969). Samples (250 μL) were mixed with 250 μL of 1 M sodium bicarbonate and 500 μL of 1% TNBS and incubated in darkness at 40 °C for 75 min. Proteins were hydrolyzed with 1.5 mL of 11 N HCl at 100 °C for 2 h. After cooling, samples were mixed with 2.5 mL of distilled water, and 2.5 mL of the diluted samples was extracted twice with 2.5 mL of diethyl ether. The organic phase, where the α-trinitrophenylamino acids are dissolved, was discarded. The aqueous phase containing the ϵ-trinitrophenyllysine was measured spectrophotometrically at 415 nm. Available lysine was quantitated using a standard curve of L-lysine monohydrochloride.

In Vitro Protein Digestibility. Solutions containing 62.5 mg of protein in 10 mL of 0.5 M NaCl were adjusted to pH 8.0. An enzymatic solution containing 1.6 mg of trypsin (17.7 BAEE U/mg), 3.1 mg of α-chymotrypsin (43 U/mg), and 1.3 mg of peptidase (50 U/g) per milliliter was added to the protein solution in a 1:10 ratio. The pH of the mixture was measured after 10 min, and the in vitro digestibility was calculated as percentage of digestible protein using the following equation: digestible protein = 210.464 − 18.103 pH (Hsu et al., 1977).

Statistical Analysis. Data were subjected to analysis of variance, and LSD value (0.05 significance) was calculated to identify pairs of means that were significantly different using the Tukey test.

RESULTS AND DISCUSSION

Linoleic Acid Oxidation. Linoleic acid is the major fatty acid in the seeds of many legumes such as soybean, chickpea, faba bean, pea, and lentil (Vioque and Maza, 1970; Sosulski and Gadan, 1988) and represents between 40 and 50% of the total fatty acids of triglycerides and phospholipids, the major lipids associated with chickpea protein isolates (Sánchez-Vioque et al., 1998a,b). Linoleic acid is highly susceptible to oxidation, either by enzymic degradation or by autoxidation, and in aqueous systems shows an oxidative stability even lower than those of fatty acids with a higher degree of unsaturation, such as linolenic or docosahexaenoic acids (Miyashita, 1993). We have observed that oxidation of linoleic acid begins during the preparation of the protein isolates, indicating the importance that this alteration might have in the further storage and processing of the product (Millán et al., 1984; Sánchez-Vioque et al., 1998a). Despite the mild conditions used for the lipid−protein incubations, linoleic acid was highly sensitive to oxidation. Thus, at the end of the incubation assay, after 48 h, 30.1% of the initial linoleic acid was oxidized. These data agree with those obtained by Miyashita et al. (1994), who found a high oxidation rate of unsaturated fatty acids from soybean when they were incubated in aqueous media under similar conditions.

Native PAGE, SDS−PAGE, and Gel Filtration Liquid Chromatography of the Incubation of Legumin with Linoleic Acid. The electrophoretic pattern under nondenaturing conditions of legumin was characterized by a major band with a relative mobility of 0.25 and two minor bands (Figure 1). The minor bands could be aggregates of legumin because this protein easily polymerizes (Wolf and Nelsen, 1996; Gruener and Ismond, 1997). Protein damage as a result of prooxidant agents, such as oxidized lipids, is usually revealed by electrophoretic analyses. Native PAGE showed that legumin was highly modified after the incubation with linoleic acid. The original band of legumin observed in the control disappeared, and bands with higher relative mobility than the native legumin appeared (Figure 1). Similar changes in the electrophoresis migration have been observed in oxidized proteins of apple juice. Electrophoretic patterns of the apple samples were characterized by a band smearing, also observed in chickpea legumin, that increased with the oxidation of the proteins (Wall et al., 1996). Other changes caused by oxidized lipids on protein structure are the fragmentation of proteins (Matoba et al., 1984; Castilho et al., 1996) and the cross-linking of protein chains caused by hydroperoxides (Kawasaki and Ooizumi, 1996) or by secondary products from lipid oxidation (Chio and Tappel, 1969). The SDS−PAGE patterns of the control and legumin incubated with linoleic acid were identical (data not shown), suggesting that there is no breakage of peptidic bonds, although breakage of disulfide bridges or noncovalent bonds is possible. To confirm this, we have developed gel filtration analysis of the samples. The breakage and polymerization of legumin were discarded as possibilities after the samples had been analyzed by gel filtration chromatography

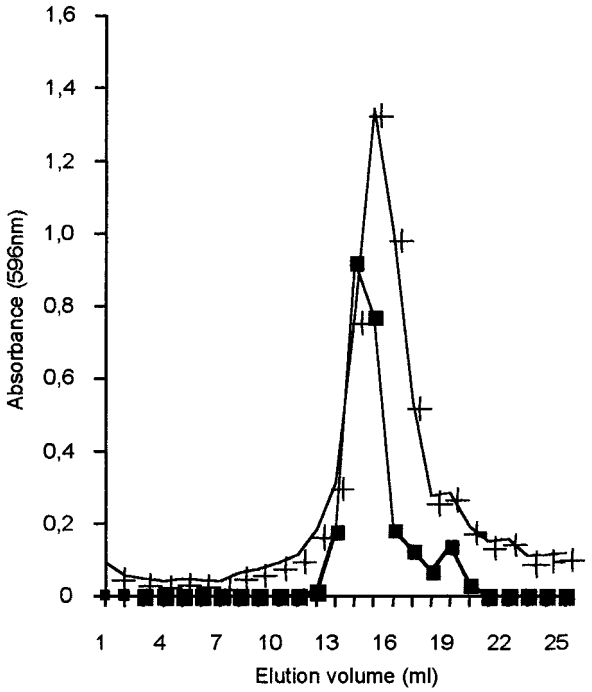

Figure 2. Gel filtration profiles of the control (■) and legumin plus linoleic acid (+) after incubation at pH 9.0 and 37 °C for 48 h. Absorbance at 596 nm was determined by Bradford's reagent.

because no differences were observed between the profiles of the control and the legumin incubated with linoleic acid. Both samples had a major peak with an elution volume (14−15 mL) corresponding to legumin (Figure 2). The increased absorbance at 16−17 mL could be related to breakage of legumin, but this possibility was rejected because it was also observed in the control. Besides, SDS−PAGE did not show bands corresponding to this molecular weight.

These results showed that the molecular weight of legumin was maintained during the incubation and that the changes observed by native PAGE are not due to cleavage of the protein. The alterations of legumin are probably due to the modification of other parameters that affect the migration in native PAGE, such as the net charge or the conformation of the protein.

Amino Acid Analysis of Chickpea Legumin. Amino acid composition may be also affected as a result of the interaction of proteins with oxidized lipids. Thus, after the incubation of chickpea legumin with linoleic acid, the methionine content, as determined by alkaline hydrolysis, was reduced significatively ($P < 0.05$) from 1.6 to 0.3 g per 100 g of protein (Table 1). This loss is probably due to the conversion of methionine to its sulfoxide, because the latter is usually formed from the reaction of proteins with oxidized lipids (Cuq et al., 1978; Nielsen et al., 1985). Moreover, when legumin incubated with linoleic acid was hydrolyzed with HCl, the methionine content remained unchanged with respect to the control (data not shown), suggesting the oxidation of this amino acid to methionine sulfoxide, and the subsequent conversion to methionine during the acid hydrolysis, as has been reported by Ray and Koshland (1960). Losses of methionine residues are especially dramatic because legumin is relatively poor in this amino acid (FAO, 1991). Histidine was also affected by oxidized linoleic acid, and its content decreased significatively ($P < 0.05$) from 3.7 to 2.8 g per 100 g of protein

Table 1. Amino Acid Composition (Grams per 100 g of Protein) of the Control and Legumin plus Linoleic Acid after Incubation at pH 9.0 and 37 °C for 48 h[a]

amino acid	FAO[b]	control	legumin + linoleic acid
aspartic acid + asparagine		13.6 ± 0.3	12.9 ± 0.2
glutamic acid + glutamine		22.4 ± 0.2	23.0 ± 0.1
serine		7.2 ± 0.1	7.6 ± 0.1
histidine	1.9	3.7 ± 0.1	2.8 ± 0.1[e]
glycine		4.5 ± 0.1	4.6 ± 0.1
threonine	3.4	3.5 ± 0.1	3.4 ± 0.1
arginine		17.1 ± 0.1	17.7 ± 0.1
alanine		4.3 ± 0.1	4.4 ± 0.1
tyrosine	6.3[c]	3.2 ± 0.1	3.2 ± 0.1
valine	3.5	4.3 ± 0.1	4.3 ± 0.1
methionine	2.5[d]	1.6 ± 0.0	0.3 ± 0.0[e]
cysteine		1.1 ± 0.1	1.1 ± 0.1
isoleucine	2.8	5.1 ± 0.1	5.2 ± 0.1
leucine	6.6	8.8 ± 0.2	9.2 ± 0.1
phenylalanine		7.5 ± 0.2	7.8 ± 0.1
lysine	5.8	5.9 ± 0.1	6.0 ± 0.1

[a] Data are the mean ± standard deviations of two replicated experiments. [b] FAO/WHO (1991) requirements for 2−5-year-old children. [c] Tyrosine + phenylalanine. [d] Methionine + cysteine. [e] Significantly different ($P < 0.05$) from their respective controls.

after the incubation. Like methionine, histidine is another amino acid that is easily oxidized. Recently, 2-oxohistidine, a compound derived from the oxidation of histidine, has been identified in several proteins subjected to oxidative conditions (Lewisch and Levine, 1995). The losses in methionine and histidine cannot be related to the incubation conditions, because the amino acid composition of the control was identical to that of legumin without incubation.

On the contrary, lysine, an amino acid normally damaged by oxidized lipids, seemed not to undergo modification, and the contents of total lysine were not decreased with respect to the control. Also, available lysine was the same for the control and the incubated legumin. Lysine residues are mainly affected by the reaction with secondary products from lipid oxidation, such as aldehydes and ketones, whereas the major losses of methionine and histidine are a consequence of their oxidation with peroxidation processes (Hidalgo et al., 1992). Therefore, this selective degradation of amino acids of legumin could be due to a predominance of primary oxidation compounds with respect to the secondary products.

In Vitro Protein Digestibility. Protein digestibility is another nutritional characteristic that may be affected upon interaction of protein with the oxidation products from lipids. We have observed that oxidation of linoleic acid lowered the in vitro protein digestibility of legumin to a large extent. After 48 h of incubation with linoleic acid, the in vitro protein digestibility of legumin was reduced from 84.1 to 69.2%. Studies on the negative effects of oxidized lipids on protein digestibility are very scarce, although these phenomena have been related to the formation of protein polymers that are minimally hydrolyzed by the enzymes (Kamin-Belsky et al., 1996). Nevertheless, Figures 1 and 2 show that legumin incubated with linoleic acid did not form aggregates; therefore, the loss of digestibility of legumin might be attributed to the changes that we have previously observed in the protein, namely, an alteration of the electrophoresis pattern and the losses of methionine and histidine. These modifications could be related to an oxidation of legumin that affects the enzymic hydrolysis of the protein. Another explanation for the

decrease of digestibility of legumin could be the possible formation of noncovalent interactions between the protein and the lipid that hinder the accessibility of the proteases to the protein.

The physicochemical conditions used in the incubations, namely, contact with the air and solubilization of the protein in an alkaline medium, are similar to those used for the preparation of the protein isolates (Sánchez-Vioque et al., 1998a). This oxidation might influence the nutritional properties of the protein isolate. The losses of essential amino acids, especially methionine, and the decrease of the protein digestibility may represent a handicap for the utilization of chickpea isolates in foods. Therefore, special attention should be paid to prevent the oxidation of lipids during the preparation of the protein isolates or its application in the elaboration of foods rich in lipids, such as sauces, cheeses, or mayonnaises. In this sense, the use of antioxidants during the preparation of the protein isolates may be a helpful strategy to minimize the damage of the proteins.

ABBREVIATIONS USED

DTT, dithiothreitol; EDTA, ethylendiaminetetraacetic acid, disodium salt; HPLC, high-performance liquid chromatography; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; TNBS, 2,4,6-trinitrobenzenesulfonic acid; Tris, tris(hydroxymethyl)-aminomethane.

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