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Effect of cooking on protein quality of chickpea (*Cicer arietinum*) seeds

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Amino acid composition and *in vitro* protein digestibility of cooked chickpea were determined and compared to raw chickpea seeds. Heat treatment produced a decrease of methionine, cysteine, lysine, arginine, tyrosine and leucine, the highest reductions being in cysteine (15%) and lysine (13.2%). Protein content declined by 3.4% and *in vitro* protein digestibility improved significantly from 71.8 to 83.5% after cooking. The decrease of lysine was higher in the cooked chickpea seeds than in the heated protein fractions, globulins and albumins. The structural modification in globulins during heat treatment seems to be the reason for the increase in protein digestibility, although the activity of proteolytic inhibitors in the albumin fraction was not reduced. Results suggest that appropriate heat treatment may improve the bioavailability of chickpea proteins.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important and cheap source of vegetable protein which can be used as a substitute for animal protein and substantially contribute to the human diet in several developing countries. The protein content of chickpea seeds is highly variable and determined by both genetic and environmental factors. Chickpea seed contains between 14.9 and 30.6% crude protein (Chavan *et al.*, 1986). Characterisation of the different protein fractions of chickpea seed has been achieved. Globulins are the storage proteins, the major fraction being in the cotyledons (Singh *et al.*, 1988). The albumin fraction is mainly compounded of enzymes and anti-nutritional factors, playing an important biological role in legume seeds. Like other legumes, the amino acid profile of chickpea is characterised by a low concentration of sulphur amino acids (methionine, cysteine) and relatively high amounts of lysine, with a higher sulphur amino acid content in the albumin fraction than in the globulins (Bhatty, 1982; Murray and Roxburgh, 1984).

The chemical composition and nutritive value of chickpea proteins are both affected by processing methods (Singh, 1985). Chickpea seed is processed and cooked in a variety of forms depending upon traditional practices and taste preferences. Different domestic processing methods (decortication, soaking, sprouting, fermentation, boiling, roasting, parching, frying, steaming) remove anti-nutritional factors and increase the protein digestibility of chickpea seed (Attia *et al.*, 1994). Data are scarce for the effect of heating on the nutritive quality of chickpea proteins. Gonzalez *et al.* (1960) reported a decrease of certain amino acids, especially lysine, cystine and arginine, when chickpea seeds are cooked. Increasing the time and temperature of cooking was reported to reduce the availability of lysine in chickpea seed (Rama Rao, 1974). To minimise amino acid losses, cooking of chickpea in an autoclave (121°C) for 1 h has been suggested (Youseff, 1983). An increase of *in vitro* protein digestibility of legume seeds after heat treatment has been reported, probably resulting from protein denaturation and inactivation of protease inhibitors (Tan *et al.*, 1984; Khokhar and Chauchan, 1986; Salunke and Kadam, 1989). Geervani and Theophilus (1980) observed a significant improvement of biological quality of chickpea protein when seeds were processed. However, in spite of the general positive effect of cooking, the final protein digestibility seems to depend on the type of process applied (Barampama and Simard, 1994).

A target is to verify the influence of heating on amino acid composition and *in vitro* protein digestibility of chickpea seed. The *in vitro* digestibility of native and processed protein fractions, globulins and albumins, has been evaluated in relating to which proteins were responsible for the reduced nutritional value.

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MATERIALS AND METHODS

Materials

Kabuli chickpea (cv Athenas) was selected for the study. Seeds were cleaned and freed from broken seeds, dust and other foreign materials. The enzymes (trypsin[porcine pancreatic trypsin type IX, 17.700 BAEE U mg^{-1}], chymotrypsin[bovine pancreatic chymotrypsin type II, 43 U mg^{-1}] and peptidase [porcine intestinal peptidase grade III, 50 Ug^{-1}]), amino acid kit, D,L-α-aminobutyric acid, and chlorogenic acid were purchased from Sigma Chemical Co. (St Louis, MO, USA).

Sample processing

Unsoaked seeds were cooked in distilled water using a seed:water ratio of 1:25 (w/v). They were processed in a digester system (1009 Digester Tecator, Hänägas, Sweden) at 120°C under pressure for 50 min, until soft. Sensorial hardness was determined by five panel members.

Homogenate flours of chickpea seeds were obtained using a domestic electric blender. Flours were defatted with hexane for 12 h in a Soxhlet apparatus, lyophilised and stored in screw-capped bottles. Extraction and fractionation of chickpea proteins was performed as described by Singh *et al.* (1988). Chickpea flour (50 g) was extracted with 500 ml 0.1 M phosphate buffer, 0.5 M NaCl, pH 7.0, by shaking at 37°C in a water bath for 1 h, centrifuged at 10 000 *g* for 30 min and the residue extracted three times with 200 ml of the same buffer. The four supernatants were pooled and dialysed against 25 mM sodium citrate buffer (pH 4.6) for 36 h and centrifuged at 25 000 *g* for 10 min. The supernatant and pellet obtained were the albumin and globulin fractions, respectively. They were frozen at −20°C, lyophilised and stored until used. Protein fractions were processed similarly to raw material. All the assays were performed in triplicate.

Total nitrogen and non-protein nitrogen determination

Homogenate flours of uncooked and cooked chickpea seeds were lyophilised and samples (0.1 g) were used for the determination of total and non-protein nitrogen by the micro-Kjeldahl method (AOAC, 1975). Crude protein content was calculated using a factor of 6.25. An extraction with 70% ethanol was carried out for determination of non-protein nitrogen (Bhatty, 1973).

In vitro protein digestibility

In vitro protein digestibility (IVPD) was evaluated according to Hsu *et al.* (1977). The protein solutions, 6.25 mg ml^{-1} in distilled water, were adjusted to pH 8.0 with 0.1 N NaOH while stirring at 37°C in a water bath. The enzyme mixture (1.6 mg trypsin; 3.1 mg chymotrypsin and 1.3 mg peptidase ml^{-1}) was maintained in an ice-bath and adjusted to pH 8.0 with 0.1 N NaOH. The multienzyme solution was added to the protein solution at a ratio of 1:10 (v/v). The pH decrease was recorded over a 10-min period with a pH meter. Percentage protein digestibility (Y) was then calculated from the equation $Y = 210.464 - 18.10X$, where X is the pH change after 10 min (Hsu *et al.*, 1977).

Nitrogen solubility

To determine nitrogen solubility of raw and cooked material, a 1:20 (w:v) seed flour:buffer (0.1 M phosphate buffer, pH 7.0, 0.5 M NaCl) ratio was used. Flour was stirred for 5 h at 25°C and centrifuged at 8000 *g* for 15 min. Supernatant was analysed for nitrogen by the micro-Kjeldahl method.

Amino acids analysis

Samples (10 mg) were hydrolysed with 4 ml 6 N HCl. The solutions were sealed in hydrolysis tubes under nitrogen and incubated in an oven at 110°C for 24 h. Amino acids were determined in the acid hydrolysis after derivatisation with diethylethoxymethylenemalonate by reverse phase-high pressure liquid chromatography (HPLC), according to the method of Alaiz *et al.* (1992), with D,L-α-aminobutyric acid as internal standard. Losses of amino acids, sensitive to acid hydrolysis, especially methionine and cysteine, were considered for accurate quantification. The HPLC system consisted of a Model 600E multi-system with a 484 UV–vis detector (Waters, Milford, MA). Separations were obtained with a 300×3.9 mm I.D. reversed phase column (Nova-Pack C_{18}, 4 μ Waters) using a binary gradient system. The solvents used were: (A) 25 mM sodium acetate containing 0.02% sodium azide (pH 6.0) and (B) acetonitrile. Solvent was delivered to the column at a flow-rate of 0.9 ml min^{-1} as follows: time 0.0–3.0 min, linear gradient from A:B (91:9) to A:B (86:14); 3.0–13.0 min, elution with A:B (86:14); 13.0–30.0 min, linear gradient from A:B (86:14) to A:B (69:31); 30.0–35.0 min, elution with A:B (69:31). The column was maintained at 18°C by a temperature controller (Julabo F10).

Polyphenols analysis

Five grammes of the fat-free flour was extracted for 8 h with 100 ml 80% ethanol in a Soxhlet extractor and the extracted volume was reduced to 25 ml under vacuum at 40°C. Samples were filtered through No.1 Whatman paper and absorption measurements at 324 nm were carried out in a spectrophotometer (Beckman DU 640, Fullerton, CA). Amounts of phenolic compounds were estimated as chlorogenic acid equivalents (Moores *et al.*, 1948).

Table 1. Losses of protein (%) during cooking in chickpea seed

Cooking time (min)	Sensorial hardness	Losses of protein content
10	very hard	ND
20	hard	ND
30	partially soft	0.6 ± 0.1
40	soft	2.8 ± 0.2
50	cooked	3.4 ± 0.4

Data refered to 100% of protein in the uncooked seeds. Each value is the mean ± SD of three independent determinations. ND, not detected.

Statistical analysis

Data were subjeted 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

Protein content

Chickpea seed protein and non-protein nitrogen content in cv Athenas were 18.9 and 0.2%, respectively. The globulin fraction (64.2%) was the main one, followed by albumins (16.0%). Different studies have reported that globulins are the major seed protein, ranging between 60 and 80%, and the albumin fraction from 12 to 23% (Singh, 1985; Singh *et al.*, 1988). Such variations may be attributable to genetic differences, environmental factors and methods of protein extraction of the sample.

A significant reduction of 3.4% in protein content was observed when the seeds were cooked (Table 1). Attia *et al.* (1994) observed similar declines in protein content (1.3–4.1%) after cooking. The presence of proteins in the processing water after cooking may be due to the partial breakage of chickpea seed and subsequent leaching of the proteins into the cooking water.

Amino acid composition

The amino acid composition of raw chickpea (*Cicer arietinum* L.) seed has been reported (Singh *et al.*, 1981). Table 2 shows the total amino acid composition of raw and cooked chickpea seed for cv Athenas in comparison with the FAO/WHO reference pattern of essential amino acids (FAO/WHO/ONU, 1985). The levels of methionine (1.75 g/100 g raw protein) and cysteine (1.54 g/100 g raw protein) were not limiting, although Singh *et al.* (1981) reported that sulphur-containing amino acids were limiting in chickpea seeds. Raw chickpea seed showed a high content of lysine (8.28 g/100 g raw protein) supporting the results of other authors (Chavan *et al.*, 1986; Singh *et al.*, 1988). We observed high amounts of arginine, aspartic and glutamic acids in chickpea seed, these three amino acids accounting for 44.22 g/100 g of the raw protein. The chickpea is very important for feeding the population in several developing countries and could be used as supplement for lysine deficient proteins.

Essential amino acid to total amino acid ratio (E/T%) was significantly ($P \leqslant 0.05$) higher for raw chickpea (41.8%) than cooked seed (39.3%). The heat treatment produced a decrease of methionine, cysteine,

Table 2. Amino acid composition[a] and true protein[b] of raw and cooked chickpea seed

Amino acid	Raw seed	Cooked seed	FAO pattern[c]
Aspartic acid	12.9 ± 0.31	12.8 ± 0.26	
Glutamic acid	18.1 ± 0.27	18.0 ± 0.31	
Serine	6.60 ± 0.23	6.60 ± 0.15	
Histidine	2.94 ± 0.12	2.81 ± 0.10	
Glycine	4.40 ± 0.08	4.33 ± 0.07	
Threonine	4.51 ± 0.11	4.34 ± 0.13	3.4
Arginine	13.2 ± 0.29	12.4 ± 0.26[d]	
Alanine	4.30 ± 0.07	4.21 ± 0.10	
Tyrosine	3.30 ± 0.11	3.12 ± 0.14	
Valine	4.83 ± 0.14	4.54 ± 0.12	3.5
Methionine	1.75 ± 0.05	1.62 ± 0.04	2.5[e]
1/2 Cystine	1.54 ± 0.09	1.31 ± 0.06[d]	
Isoleucine	5.04 ± 0.08	4.87 ± 0.10	2.8
Leucine	9.32 ± 0.14	8.98 ± 0.16[d]	6.6
Phenylalanine	6.34 ± 0.10	5.91 ± 0.11[d]	6.3[f]
Lysine	8.28 ± 0.14	7.19 ± 0.19[d]	5.8
True protein (%)	18.9 ± 0.50	18.3 ± 0.08	
Non-protein nitrogen (%)	0.20 ± 0.02		

Each value is the mean ± SD of three determinations.
[a]Grammes of amino acid/100 g protein.
[b](Total nitrogen–non-protein nitrogen) × 6.25.
[c]FAO/WHO/ONU (1985).
[d]Significantly different ($P < 0.05$) from their respective controls.
[e]Met + Cys.
[f]Tyr + Phe.

Table 3. Amino acid composition[a] of albumin and globulin fractions in their native state and after heating

Amino acid	Native globulin	Heated globulin	Native albumin	Heated albumin	FAO pattern[b]
Aspartic acid	12.9 ± 0.23	12.8 ± 0.16	13.0 ± 0.10	13.00 ± 13.0	
Glutamic acid	19.8 ± 0.17	19.7 ± 0.21	16.9 ± 0.14	16.8 ± 0.09	
Serine	5.24 ± 0.12	5.21 ± 0.14	4.74 ± 0.10	4.41 ± 0.10[c]	
Histidine	2.91 ± 0.07	2.83 ± 0.10	2.75 ± 0.14	2.66 ± 0.10	
Glycine	4.21 ± 0.14	3.94 ± 0.15	5.43 ± 0.12	5.31 ± 0.08[c]	
Threonine	3.47 ± 0.09	3.42 ± 0.06	6.04 ± 0.08	5.73 ± 0.08	3.4
Arginine	13.9 ± 0.21	13.6 ± 0.17	8.24 ± 0.12	8.07 ± 0.14	
Alanine	4.21 ± 0.14	4.20 ± 0.11	4.86 ± 0.10	4.81 ± 0.15	
Tyrosine	2.99 ± 0.03	2.91 ± 0.05	3.74 ± 0.07	3.73 ± 0.07	
Valine	5.54 ± 0.14	5.52 ± 0.09	5.22 ± 0.10	5.15 ± 0.08	3.5
Methionine	1.72 ± 0.07	1.61 ± 0.10	3.24 ± 0.11	3.14 ± 0.09	2.5[d]
1/2 Cystine	1.01 ± 0.08	0.72 ± 0.11[c]	2.84 ± 0.14	2.63 ± 0.10[c]	
Isoleucine	5.73 ± 0.14	5.61 ± 0.07	5.34 ± 0.10	4.93 ± 0.06[c]	2.8
Leucine	9.42 ± 0.26	9.21 ± 0.14	7.47 ± 0.12	6.84 ± 0.08[c]	6.6
Phenylalanine	6.67 ± 0.11	6.54 ± 0.11	4.93 ± 0.10	4.45 ± 0.07[c]	6.3[e]
Lysine	7.67 ± 0.09	7.21 ± 0.07[c]	10.8 ± 0.06	9.95 ± 0.05[c]	5.8

Each value is the mean ± SD of three independent determinations.
[a]Grammes of amino acid/100 g protein.
[b]FAO/WHO/ONU (1985).
[c]Significantly different ($P < 0.05$) from their respective controls.
[d]Met + Cys.
[e]Tyr + Phe.

lysine, arginine, tyrosine and leucine, reductions in cysteine (15.0%) and lysine (13.2%) being the highest. Youseff (1983) reported similar losses in lysine while Geervani and Theophilus (1980) found a very important reduction of sulphur amino acids (22–26%). These figures appear to be higher than the values reported in this study. This can be explained by the excessive heat treatment used in the cooking experiments. Heat treatment causes considerable nutritional damage to methionine (Shemer and Perkins, 1975) and cysteine, the most limiting essential amino acids in legume seeds. Therefore, damage caused by heat treatment in sulphur amino acids could be considered a quality control parameter in the cooking process of legumes.

With respect to the major protein fractions, albumins contained significantly higher levels of sulphur amino acids (6.08 g/100 g raw protein) and lysine (10.78 g/100 g raw protein) than globulins (2.73 and 7.67 g/100 g raw protein, respectively). The effect of heat treatment was investigated in globulins and albumins in their native state after extraction and fractionation (Table 3). Losses of lysine (13.2%) in cooked chickpea seeds were significantly higher than in heated albumin (7.7%) and globulin (6.0%) fractions. This might be attributed to possible reactions (Maillard reactions) between the free ε-amino group and the reducing components in chickpea seeds. In order to reduce the losses of essential amino acids, mainly sulphur amino acids, it is necessary to be sure that heating temperature and processing time reach, and do not exceed, the optimum required, because prolonged cooking of legumes may result in destruction and racemisation of amino acids (Salunke *et al.*, 1985). Also, excessive heating may reduce the nutritive value of protein, possibly by promoting amide cross-linkage of amino acid side-chains (Shemer and Perkins, 1975).

Table 4. Effect of cooking on *in vitro* protein digestibility and protein solubility of chickpea seed

Sample	Digestibility[a]	Solubility
Raw seed	71.8 ± 1.0	43.0 ± 3.0
Cooked seed	83.5 ± 0.1[b] (+14.1)	3.9 ± 0.2 (−91.0)
Native globulins	76.1 ± 1.0	—
Heated globulins	81.1 ± 0.4[b]	—
Native albumins	69.7 ± 0.4	—
Heated albumins	70.6 ± 0.1	—

[a]Protein hydrolysed as percentage of total proteins. Values are mean ± SD of three independent determinations. Figures in parentheses represent percentage increase (+) or decrease (−) over raw flour.
[b]Significantly different ($P < 0.05$) from the raw seed.

Table 5. Decrease in polyphenol content (g kg^{-1}chickpea seed) during cooking in chickpea seeds

Cooking time (min)	Sensorial hardness	Polyphenols content in chickpea seeds	Losses (%)[a]
0	—	2.14 ± 0.12	—
10	very hard	1.91 ± 0.01	10.8
20	hard	1.77 ± 0.02	17.2
30	partially soft	1.48 ± 0.05	30.6
40	soft	1.16 ± 0.03	45.8
50	cooked	1.06 ± 0.03	50.2

Data represent mean ± SD of three independent determinations.
[a]Percentage refers to total content of polyphenols in chickpea seed.

In vitro protein digestibility

The biological utilisation of a protein is primarily dependent on its digestibility by proteases. Heat treatment may alter the protein structure leading to changes in the digestibility. An *in vitro* method was used to assay digestibility. In comparison with *in vivo* methods, these are reliable, rapid, simple and could be used commercially for monitoring protein quality (Swaisgood and Catignani, 1991).

A significant ($P \leqslant 0.05$) increment of *in vitro* protein digestibility, from 71.8 to 83.5%, was observed when chickpea seeds were cooked (Table 4). The proteolytic resistance of raw chickpea proteins may be attributed to structural characteristics of the globulin fraction, its intracellular location, the presence of digestive enzyme inhibitors and other anti-nutritional factors such as polyphenols (Melito and Tovar, 1995).

The *in vitro* protein digestibility of the globulin fraction (76.1%) was higher than in the unprocessed whole seed (71.8%). This suggests that protein structure is not the unique factor affecting protein digestibility in chickpea seed.

The low digestibility of the albumin fraction (69.7%) could be explained by the reported presence of trypsin and chymotrypsin inhibitors (Singh and Jambunathan, 1981). Besides, heat treatment did not significantly increase the *in vitro* protein digestibility of albumins (70.6%), suggesting that protease inhibitors are not affected by the treatment.

The considerable reduction in nitrogen solubility of cooked seeds with respect to raw material (Table 4) could be due to the denaturation of globulins after heating. This denaturation makes proteins more susceptible to proteolysis, increasing the total *in vitro* protein digestibility after cooking.

A highly significant negative correlation between *in vitro* protein digestibility and total phenolic compounds of chickpea seeds has been reported (Singh and Jambunathan, 1981). Phenols react with proteins forming poorly extractable protein–phenolic complexes, leading to enzymic inhibition and consequently lower protein digestibility (Jood *et al.*, 1987). Removal of polyphenols is necessary for effective utilisation of chickpea seeds for human nutrition. In cooked chickpea, lower levels of polyphenols as compared to raw chickpea seeds were observed (Table 5). The increment of *in vitro* protein digestibility after heat treatment may be partially a consequence of leaching into the cooking water of polyphenols under the influence of a concentration gradient (Jood *et al.*, 1987, Lanfer Marquez and Lajolo, 1990).

The results reported show that chickpea processing is advisable to improve its nutritional quality because of the increase of *in vitro* protein digestibility and the reduction in polyphenol content.

On the other hand, it is necessary to optimise the cooking process to reduce the decrease in the contents of essential amino acids. Further studies are needed to determine the structural changes of chickpea proteins and the interactions with other compounds during the cooking process.

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