Nutritional Assessment of Raw and Processed Chickpea (*Cicer arietinum* L.) Protein in Growing Rats

Teresa Nestares, Magdalena López-Frías,* Mercedes Barrionuevo, and Gloria Urbano

Department of Physiology, School of Pharmacy, and Institute of Nutrition and Food Technology, University of Granada, E-18071 Granada, Spain

We studied the digestive and metabolic utilization of chickpea protein (*Cicer arietinum* L.) from raw and processed chickpeas (dry-heated, soaked in distilled water or an acid, or a base solution, and soaked + cooked). Chemical and biological methods were used for nutritional determinations in growing rats. Food intake, calculated as a function of body weight, was higher for processed than for raw chickpeas. The digestibility of chickpea protein was not affected by soaking, but was increased after soaking + cooking. This effect may be related to reduced trypsin inhibitor activity and tannin content. Nitrogen retention (nitrogen balance) was better after soaking in basic medium without cooking and after soaking + cooking regardless of the pH of the soaking medium. However, nitrogen balance was lower than expected from the chemical analyses of the protein in the different diets. Soaking in basic medium with or without cooking led to the highest food intake, nutritive utilization of protein, and weight gain. The faster rate of growth was probably due to the improved utilization of carbohydrates in chickpeas soaked in basic medium.

Keywords: Chickpeas; nutritive utilization; protein; processing techniques

INTRODUCTION

Chickpea (Cicer arietinum L.) is a good source of protein, carbohydrates, calcium, and phosphorus, among other compounds. It is the most widely consumed legume throughout Spain and is especially popular in Andalusia (Varela et al., 1995). Together with other legumes, it has long been one of the most important sources of protein in rural populations. Chickpea is also widespread in Asia and Central and South America, where is satisfies a considerable portion of the population's protein requirements. Although the seed is rich in protein (about 21.5%; Chavan et al., 1989), both the quantity and the quality of protein vary considerably depending on soil and climatological conditions (location, agricultural practices) (Singh et al., 1983; Rossi et al., 1984). This makes is necessary to investigate different cultivars grown in different regions individually.

Like other pulses, chickpeas contain several antinutritional factors (α -galactosides, trypsin inhibitors, tannins, etc.) which may limit their consumption and the nutritive utilization of their protein. These antinutritional factors can be eliminated or reduced by cooking or with other simple technologies (Nestares et al., 1993a; Vidal et al., 1994; Urbano et al., 1995), although processing will modify the nutritive utilization of protein. These changes differ widely depending on the technology and conditions involved (Nestares et al., 1993a; Urbano et al., 1995).

The objective of this study was to evaluate the nutritional quality of a chickpea cultivar habitually grown and consumed in southern Spain. In addition, we investigated how different, commonly used processing techniques affect the nutritive utilization of chickpea protein and the antinutritional factors that influence this utilization. To remove heat-sensitive antinutritional factors, we used dry-heating under pressure. Thermostable factors were removed by soaking chickpeas in distilled water or acid or basic medium and by soaking followed by cooking.

MATERIALS AND METHODS

Samples. Raw, dried chickpeas (R) (*Cicer arietinum* L.) were grown in Andalusia (Southern Spain). The seeds were subjected to seven different treatments: H = dry heating, S = soaking in distilled water, SA = soaking in acid medium, SB = soaking in basic medium, SC = S + cooking, SAC = SA + cooking, SBC = SB + cooking.

Processing Techniques. *Heating.* Raw chickpeas were dry-heated under presure at 120 °C, 1 atm, for 15 min.

Soaking. In processes S, SA, and SB, raw seeds were soaked at room temperature for 9 h in distilled water (pH = 5.3), citric acid solution (0.1%, pH = 2.6), or sodium bicarbonate solution (0.07%, pH = 8.4). The seed to solution ratio was 1:3 (wt:vol). The soaking liquid was drained off, and the seeds were blended and lyophilized.

Cooking. Soaked chickpeas were cooked (SC, SAC, SBC) by boiling in distilled water for 35 min, at a seed to water ratio of 1:6.67 (wt:vol). The cooking water was drained off, and the seeds were crushed and lyophilized.

Analytical Techniques. Nitrogen was determined with the method of Kjeldahl. The protein conversion factor was 6.25.

Water content was determined by oven-drying at 105 \pm 1 $^\circ\mathrm{C}$ until a constant weight was obtained.

Protein nitrogen content was determined in protein precipitated with copper acetate.

The amino acid composition of the proteins was determined by high-performance liquid chromatography (Pico-Tag method) of acid-digested samples; cysteine and methionine were analyzed after performic acid oxidation.

Biological Methods. *Experimental Design and Diet.* We used a biological balance technique, recorded food intake, and changes in body weight and calculated nitrogen intake and fecal and urinary nitrogen excretion.

Eight experiments were done in which raw or processed chickpeas were the only source of food: group R, raw chickpeas; group H, chickpeas dry-heated under pressure; group S, chickpeas soaked in distilled water; group SB, chickpeas

^{*} Address correspondence to this author at Depto. de Fisiología, Facultad de Farmacia, Campus de Cartuja, Universidad de Granada E-18071 Granada, Spain (telephone +34-58-243879; fax +34-58-243879).

Table 1. Composition of Nitrogen of Raw and Processed Chickpeas in Dry Matter

	-	-		-	•		
diet ^a	crude protein (%)	total N content (%)	nonprotein N content ^b	diet ^a	crude protein (%)	total N content (%)	nonprotein N content ^b
R	21.2	3.38	8.81	SB	22.9	3.65	8.93
Н	22.4	3.58	8.75	SC	23.0	3.67	8.89
S	21.8	3.50	8.61	SAC	24.1	3.86	9.09
SA	22.5	3.58	8.82	SBC	23.8	3.81	9.02

^{*a*} R, raw chickpeas; H, heated chickpeas; S, soaked chickpeas; SA, chickpeas soaked in acid medium; SB, chickpeas soaked in basic medium; SC, soaked and cooked chickpeas; SAC, chickpeas soaked in acid medium and cooked; SBC, chickpeas soaked in basic medium and cooked. ^{*b*} As percent of total nitrogen content.

Table 2. A	Amino Acid (AA)	Composition of Rav	v and Processed Chick	oeas (Grams per 16	g of Nitrogen,	in Dry Matter) ^a

	· •			-	• •	0	0	
amino acid	R	Н	S	SA	SB	SC	SAC	SBC
Asp	2.23	2.38	1.94	2.37	2.34	2.38	2.32	2.41
Glû	3.83	4.11	4.02	4.04	4.05	4.10	4.15	4.16
Ser	0.97	1.02	1.01	1.02	1.03	1.05	1.13	1.07
Gly	0.94	0.99	1.00	0.99	1.03	1.02	1.11	1.08
His	0.29	0.30	0.37	0.31	0.30	0.32	0.32	0.33
Thr	0.77	0.84	0.81	0.85	0.85	0.82	0.90	0.88
Ala	0.99	0.98	0.91	1.00	1.02	1.05	1.08	1.13
Arg	1.83	1.88	2.05	2.01	2.09	2.08	2.15	2.13
Pro	0.98	1.12	1.31	1.12	1.17	1.20	1.32	1.28
Val	1.46	1.39	1.31	1.42	1.44	1.46	1.57	1.52
Ile	1.17	1.22	1.17	1.27	1.24	1.29	1.36	1.31
Leu	2.13	2.29	2.16	2.31	2.35	2.40	2.52	2.47
Phe	1.39	1.45	1.50	1.41	1.47	1.47	1.68	1.58
Lys	1.42	1.57	1.46	1.46	1.61	1.50	1.77	1.60
Met	0.33	0.36	0.39	0.36	0.38	0.38	0.41	0.42
Cys	0.41	0.46	0.46	0.45	0.45	0.43	0.36	0.44
total AA (%)	21.13	22.38	21.88	22.38	22.81	22.94	24.13	23.81
total branched AA	4.76	4.90	4.64	5.00	5.03	5.15	5.45	5.30
total sulfurized AA	0.74	0.82	0.85	0.81	0.83	0.81	0.77	0.86

^{*a*} R, raw chickpeas; H, heated chickpeas; S, soaked chickpeas; SA, chickpeas soaked in acid medium; SB, chickpeas soaked in basic medium; SC, soaked and cooked chickpeas; SAC, chickpeas soaked in acid medium and cooked; SBC, chickpeas soaked in basic medium and cooked.

soaked in basic medium; SA, chickpeas soaked in acid medium; group SC, chickpeas soaked in bidistilled water and cooked; group SBC, chickpeas soaked in basic medium and cooked; SAC, chickpeas soaked in acid medium and cooked.

Each experiment lasted 10 days. During the first 3 days the rats were allowed to adapt to the diet and experimental conditions, and the main experimental period comprised the next 7 days, during which body weight and food intake were recorded and feces and urine were collected for subsequent analysis. The diet and bidistilled water were available *ad libitum* throughout the experimental period.

Animals. In each experiment we used 10 young albino Wistar rats (5 male, 5 female), reared in the University of Granada Laboratory Animal Services. The growing animals (recently weaned), with an initial body weight of 58.8 ± 1.5 g, were housed in individual metabolic cages kept in a thermoregulated room (22 ± 1 °C) with a controlled 12 h light: dark period (lights on at 9.00).

Biological Indices. The following indices and parameters were determined for each group, according to the formulas given below: intake (expressed as dry weight), body weight, protein efficiency ratio (PER, eq 1), apparent digestibility coefficient (ADC, eq 2) for protein and nitrogen retention (nitrogen balance, eq 3), and percent nitrogen retention/ nitrogen absorption (%R/A, eq 4).

$$PER = \frac{\text{weight gained ((g/rat)/day)}}{\text{protein intake ((g/rat)/day)}}$$
(1)

$$ADC = \frac{I - F}{I} \times 100$$
 (2)

$$balance = I - (F + U)$$
(3)

$$\% R/A = \frac{I - (F + U)}{I - F} \times 100$$
 (4)

In accordance with the formulas recommended by the FAO/WHO (1966), the factors used were I (nitrogen intake), F (fecal nitrogen), and U (urinary nitrogen).

Body weight and protein intakes were expressed as (g/rat)/ day.

Statistical Methods. The results from all experiments and analyses were tested statistically by analysis of variance using Statgraphic Statistical Graphics 2.1 System software (Statistical Graphics Corp., Rockville, MD) with an IBM Personal System/2 Model 20 computer (International Business Machines Corp., North Harbour Portsmouth, U.K.).

RESULTS

Chemical Analysis. Table 1 gives the values for percent protein, total nitrogen content, and nonprotein nitrogen content in raw and processed chickpea diets. Raw chickpeas contained 3.38% total nitrogen, of which 8.81% was nonprotein nitrogen. These values were not significantly affected by any of the processing techniques tested here. The apparent increase in nitrogen concentration was due to solubilization of carbohydrates in the soaking and cooking liquids.

The amino acid composition of chickpea protein is summarized in Table 2. None of the processes used to prepare chickpeas before feeding modified the amino acid composition significantly. The total amino acid content in all diets, expressed as grams of amino acid per 100 grams of diet, was virtually the same as the protein content.

The percent relative amounts of essential amino acids in dietary proteins are shown in Table 3, referred to chicken egg albumin (FAO/WHO, 1973). The chemical score for chickpea protein showed that this figure was limited mainly by methionine, cysteine, and threonine, which received scores of 45.17, 54.91, and 91.15, respectively, in raw chickpeas. Processing had no significant effect on these values.

Biological Analysis. Food intake, expressed as grams of diet per 100 grams of body weight, was

Table 3. Chemical Score in Raw and Processed Chickpeas^a

amino acid	R (%)	H (%)	S (%)	SA (%)	SB (%)	SC (%)	SAC (%)	SBC (%)
Phen	109.53	107.98	114.57	104.63	107.12	106.43	115.77	110.25
Ile	138.25	136.63	133.68	141.30	136.23	140.70	140.70	137.85
Leu	143.94	146.43	140.77	147.46	147.30	149.21	149.37	148.37
Lys	121.91	127.64	121.49	118.62	128.09	119.13	133.67	121.80
Lys Met	45.17	46.23	51.46	45.31	47.23	47.09	48.77	50.40
Cys	54.91	58.97	59.69	57.06	56.74	54.06	42.74	52.80
Cys Val	137.78	124.12	119.92	127.16	126.34	127.38	129.72	127.34
Thr	91.15	93.73	92.90	94.73	93.38	89.03	93.15	92.40

^{*a*} R, raw chickpeas; H, heated chickpeas; S, soaked chickpeas; SA, chickpeas soaked in acid medium; SB, chickpeas soaked in basic medium; SC, soaked and cooked chickpeas; SAC, chickpeas soaked in acid medium and cooked; SBC, chickpeas soaked in basic medium and cooked.

Table 4. Food Intake and Weight Change in Rats Fed Chickpea Diets^a

group ^a	body wt gain ((g/rat)/day)	dry matter intake ((g/rat)/day)	dry matter intake ((g/100 g of rat)/day)	protein intake ((g/rat)/day)	PER ^c
R	0.88 ± 0.13^a	6.00 ± 0.22^a	9.73 ± 0.37	1.27 ± 0.05	0.57 ± 0.10^a
Н	1.01 ± 0.19^a	7.25 ± 0.23^b	10.79 ± 0.22^b	1.62 ± 0.05^a	0.63 ± 0.11^a
S	1.56 ± 0.10^b	$7.59\pm0.26^{b,c}$	$10.88 \pm 0.17^{b,c}$	$1.65\pm0.06^{a,b}$	0.96 ± 0.08^b
SA	$1.31\pm0.09^{b,c}$	6.29 ± 0.11^a	$10.56 \pm 0.18^{b,c}$	1.41 ± 0.02	$0.93\pm0.06^{b,c}$
SB	2.99 ± 0.21	8.95 ± 0.17	12.19 ± 0.25^d	$\textbf{2.04} \pm \textbf{0.04}$	1.46 ± 0.10
SC	$1.46\pm0.11^{b-d}$	$7.40\pm0.34^{b-d}$	$12.32 \pm 0.40^{d,e}$	$1.70\pm0.08^{a-c}$	$0.85\pm0.03^{b-d}$
SAC	$1.30\pm0.05^{b-d}$	$7.03\pm0.13^{b,d,e}$	$12.00 \pm 0.21^{d,e}$	$1.69\pm0.03^{a-d}$	0.77 ± 0.03^d
SBC	2.14 ± 0.19	$7.17\pm0.22^{b-e}$	11.36 ± 0.26^{c}	$1.71\pm0.05^{a-d}$	1.24 ± 0.09

^{*a*} The same superscript letter in the same column indicates no significant differences (p < 0.05). Values are means \pm SEM of 10 Wistar rats. ^{*b*} R, raw chickpeas; H, heated chickpeas; S, soaked chickpeas; SA, chickpeas soaked in acid medium; SB, chickpeas soaked in basic medium; SC, soaked and cooked chickpeas; SAC, chickpeas soaked in acid medium and cooked; SBC, chickpeas soaked in basic medium and cooked. ^{*c*} PER, weight gained ((g/rat)/day)/protein intake ((g/rat)/day).

Table 5. Digestive Utilization^a

group ^b	nitrogen intake ((mg/rat)/day)	total fecal nitrogen ((mg/rat)/day)	absorbed nitrogen ((mg/rat)/day)	ADC
R	202.80 ± 7.47	43.64 ± 4.26^a	159.16 ± 5.39	78.70 ± 1.57^a
Н	259.40 ± 8.46^a	62.93 ± 3.31^b	196.47 ± 6.70^{a}	75.75 ± 0.89
S	$263.68 \pm 9.06^{a,b}$	55.45 ± 2.67	208.23 ± 7.72^a	$78.95 \pm 0.80^{a,b}$
SA	225.92 ± 3.78	$47.45 \pm 1.81^{a,c}$	178.47 ± 3.66	$\textbf{78.98} \pm \textbf{0.77}^{\textit{a-c}}$
SB	327.04 ± 6.32	63.11 ± 3.45^b	263.93 ± 5.27	$80.74 \pm 0.90^{a-d}$
SC	$271.70 \pm 12.52^{a-c}$	$47.18 \pm 4.18^{a,c,d}$	224.52 ± 12.46^{b}	$82.46 \pm 1.45^{d,e}$
SAC	$271.10 \pm 4.89^{a-d}$	$45.79 \pm 2.22^{a,c-e}$	$225.31 \pm 4.69^{b,c}$	$83.10 \pm 0.77^{e,f}$
SBC	$273.34 \pm 8.53^{a-d}$	$42.07 \pm 1.57^{a,c-e}$	$231.27 \pm 7.79^{b,c}$	84.56 ± 0.53^{f}

^{*a*} The same superscript letter in the same column indicates no significant differences (p < 0.05). Values are means \pm SEM of 10 Wistar rats. ^{*b*} R, raw chickpeas; H, heated chickpeas; S, soaked chickpeas; SA, soaked in acid medium chickpeas; SB, soaked in basic medium chickpeas; SC, soaked and cooked chickpeas; SAC, soaked in acid medium and cooked chickpeas; SBC, soaked in basic medium and cooked chickpeas.

significantly greater for all processed diets than for raw chickpeas. The greatest increases were found in groups SB, SC, and SAC (Table 4).

Daily weight gain (Table 4) was significantly lower in rats fed with raw (group R) or dry-heated chickpeas (group H). Soaking in basic medium with (SBC) or without cooking (SB) led to significantly greater weight gains in these groups than in the others. When weight gain in grams was expressed per grams of protein ingested (PER), the results were the same (Table 4). In group SBC, the PER was twice as high as in group R; the value for group SB was almost three times as high as that for group R. Differences in protein intake were related to food intake, as there was no difference between the diets in nitrogen content.

Nitrogen absorption (Table 5) in absolute values was significantly higher in animals fed with dry-heated (H), water-soaked (S), or acid-soaked chickpeas (SA) than in group R. Cooking after soaking further increased nitrogen absorption. In rats fed with basic-soaked chickpeas, absorption was significantly higher than in all other groups. In general, we found that all types of processing improved nitrogen absorption.

The digestive utilization of protein, calculated as the ADC (Table 5), was reduced by dry-heating at 120 °C for 15 min and was not improved by soaking. However,

Table 6. Metabolic Utilization^a

group ^b	total urinary nitrogen ((mg/rat)/day)	balance ^c	%R/A ^d				
R	108.09 ± 4.37	51.07 ± 4.07^a	31.93 ± 2.24^a				
Н	136.55 ± 6.08^a	$64.41\pm8.56^{a,b}$	$32.18 \pm 3.52^{a,b}$				
S	147.52 ± 7.30^b	$60.71 \pm 2.91^{a-c}$	$29.39 \pm 1.50^{a-c}$				
SA	$125.32 \pm 6.01^{a,c}$	$53.15 \pm 4.91^{a-c}$	$29.87 \pm 2.76^{a-c}$				
SB	147.99 ± 3.83^{b}	115.94 ± 6.57	43.73 ± 1.82^d				
SC	$132.25 \pm 7.97^{a,c,d}$	92.27 ± 11.35^{d}	$40.44\pm3.57^{d,e}$				
SAC	$132.99 \pm 6.28^{a,c-e}$	$92.32\pm5.68^{d,e}$	$40.99 \pm 2.49^{d-f}$				
SBC	$130.67 \pm 6.30^{a,c-e}$	$100.60\pm8.65^{d,e}$	$43.17 \pm 2.71^{d-f}$				

^{*a*} The same superscript in the same column indicates no significant differences (p < 0.05). Values are means \pm SEM of 10 Wistar rats. ^{*b*} R, raw chickpeas; H, heated chickpeas; S, soaked chickpeas; SA, soaked in acid medium chickpeas; SB, soaked in basic medium chickpeas; SC, soaked and cooked chickpeas; SAC, soaked in acid medium and cooked chickpeas; SBC, soaked in basic medium and cooked chickpeas. ^{*c*} Balance = nitrogen intake - (fecal nitrogen + urinary nitrogen). ^{*d*} %R/A = [balance/(nitrogen intake - fecal nitrogen) × 100].

cooking significantly improved the ADC, regardless of the type of soaking solution used previously.

The nitrogen balance in groups H, S, and SA was similar to that in group R (Table 6). Basic-soaking and cooking after soaking in any of the solutions significantly improved nitrogen balance. Like nitrogen balance, the ratio of nitrogen retained to nitrogen absorbed (%R/A) was significantly higher for the basic-soaked and all three cooked diets in comparison with all other groups (Table 6).

DISCUSSION

Chemical Analyses of Protein. The chickpeas tested in these experiments had a mean protein content of 21.22%, which was close to the value of 21.5% given by Chavan et al. (1989). The slight increase in nitrogen concentration after processing reflected the loss of total carbohydrates (Vidal-Valverde et al., 1993), a phenomenon described by Savage and Thompson (1993) in chickpeas soaked in water at 12 °C for 18 h and in chickpeas cooked in water for 40 min.

The amino acid composition of the chickpeas we tested was similar to that reported by others (Singh and Jambunathan, 1981b; Chavan et al., 1989; Combe et al., 1991) for this legume. Processing did not significantly affect amino acid content, a result that contrasts with the findings of Geervani and Theophilus (1980), who noted that cooking reduced the content of some amino acids, possibly as a result of the experimental conditions these authors used.

Percent total amino acid content in each diet tested here was close to protein content. This suggests that although as much as 8.81% of the total nitrogen content was in the form of nonprotein nitrogen, this portion comprised amino acids and peptides that might have been digestible and utilizable, as postulated by Singh and Jambunathan (1981a). These authors found that nonprotein nitrogen in chickpeas accounted for 11.2% of the total nitrogen content. The highly significant correlation that Singh and Jambunathan (1981a) found between protein content and nonprotein nitrogen (r =0.802) suggests that the chickpeas we tested may have contained less nitrogen than those used by these authors.

Our chemical scores for protein in raw and processed chickpeas show that the limiting amino acids were methionine and cysteine. In an earlier study, Chavan et al. (1989) also found these to be the limiting amino acids in chickpeas, although their chemical scores for these two amino acids were higher than ours. This difference was probably due to the larger proportion of globulin in chickpeas, since this protein fraction contains the largest amounts of sulfur-containing amino acids (Singh and Jambunathan, 1982).

The high protein content of chickpeas means that this legume supplies enough essential amino acids to cover the growing rat's nutritional requirements, as specified by the National Research Council (U.S.) (1990). Nutritional requirements were calculated from food intake (Table 4) and amino acid content (Table 2) as previously reported (Nestares et al., 1993b).

The proportion of essential and nonessential amino acids in chickpea protein is approximately 53-47%. According to the reference values given in a review by Santidrian (1987) (approximately 33% essential and 66% nonessential amino acids for proteins of high nutritional value, versus 25% essential and 75% nonessential amino acids in proteins of low nutritional value), the chickpeas we studied are of moderately good nutritional value. The indispensable/dispensable amino acid ratio (I/D) was 1:2, making this source of protein appropriate for growing rats, in accordance with the findings of Stucki and Harper (1962). These researchers obtained optimal growth after weaning with feeds in which the I/D ratio was 4:1. These findings suggest that young rats fed with the chickpea protein we tested would be expected to show good growth.

The similarities in percent protein content and protein nitrogen in the chickpea we tested and other cultivars reported in earlier studies facilitated comparisons of the metabolic and digestive parameters.

Biological Analyses of Protein. Earlier research at our laboratory showed that a more accurate indication of food intake was obtained when results were expressed as grams of food consumed per 100 g of body weight, than when this parameter was expressed as grams of food ingested per rat per day (López-Frías et al., 1985). Recordings of food intake expressed with reference to body weight during the 7 day main period of the experiment showed that this value was significantly lower in rats fed with raw chickpeas than in weanling rats fed a diet adjusted to 12 or 20% protein content (casein + DL-methionine) (Nestares et al., 1993; Fernández et al., 1993). The lower food intakes in group R than in rats fed with a 12% casein-methionine diet may have been due in part to the greater protein supply from chickpeas (Harper et al., 1967; Peters and Harper, 1985). In rats, higher supplies of dietary protein increase the serum concentration of branched amino acids (Johnson and Anderson, 1982); this effect would partially account for decreased appetite (Anderson et al., 1982), either directly, by increasing brain levels of free amino acids, or indirectly, by blocking the uptake of neutral amino acids such as tryptophan and tyrosine in the brain (Tews et al., 1978). These two amino acids are precursors for the synthesis of neurotransmitters involved in appetite control.

The lower intake of the raw chickpea diet in comparison with the 20% casein-methionine diet may have been due in part to a difference in protein quality. The amino acid imbalance in chickpeas may have reduced intake (Peters and Harper, 1985) by causing large, unspecific changes in plasma and brain amino acid profiles (Tackman et al., 1990).

The lower saccharose content in raw (3.53%) and processed chickpeas (1.76-1.93%) in comparison with a standard diet (approximately 30%) (Nestares et al., 1993b) probably accounted for the lower intake of the less palatable chickpea diets.

The fat content of chickpeas is approximately 5% (Chavan et al., 1989), a value within the limits recommended by the National Research Council (1990) for growing rats. Unsaturated fat makes up 67% of the total fat content (Chavan et al., 1989); thus, in both qualitative and quantitative terms, chickpea fat satisfies the requirements for growing rats. The type and amount of fat in this food do not affect intake (Le Magnen, 1983).

The presence of antinutritional factors such as α -galactosides and tannins may also be partly responsible for the lower intake of chickpea diets in comparison with a casein diet. Raw chickpeas from the same lot as those used to prepare the diets in the present study contain 4.84% α -galactosides (0.46% raffinose, 2.70% ciceritol, 1.68% stachyose) (Nestares et al., 1993a), compounds which reduce intake in humans and animals by causing flatulence (Singh et al., 1982; Savitri and Desikachar, 1985). The tannin content of raw chickpeas (1.19 mg/ 100 g of dry weight) (Nestares et al., 1993a) also decreases intake by precipitating salivary proteins and thus interfering with swallowing (Mole, 1989).

The significantly higher intakes (expressed as grams of food per 100 g of body weight) of the processed diets (especially SB) in comparison with raw chickpeas cannot be explained by differences in amino acid content, which was not affected by heating, soaking, or cooking. The greater intakes of processed chickpeas may have been due to reductions in antinutritional factors during treatment (Nestares et al., 1993a). However, this was probably not the only cause, as under our experimental conditions we found no direct correlation between α -galactoside or tannin content and food intake. This implies that the lower intake of raw chickpeas and the greater intakes of processed diets in comparison with a control casein-methionine diet of similar (20%) or lower (12%) protein content were caused by a combination of all the factors noted above that regulate intake. In addition, greater intakes were associated with a better overall protein synthesis, and faster growth, which made these animals less susceptible to antinutritional factors.

The digestive utilization of raw chickpea protein, expressed as the ADC, was within the range reported in earlier studies for this legume (Chavan et al., 1989; Combe et al., 1991; Savage and Thompson, 1993). However, this ADC was below the figure we obtained for a 12% casein-methionine control diet (Nestares et al., 1993b). This may have been due to the presence of trypsin inhibitors $(10.43 \pm 0.77 \text{ units/mg of dry weight})$ (Nestares et al., 1993a), which impede the complete digestion of protein. Tannins, also present in the chickpeas we tested, increase the endogenous fecal excretion of nitrogen and were also partly responsible for the decrease in protein ADC. The consumption of legumes increases endogenous nitrogen loss through the shedding of intestinal mucosa (Sanoja and Bender, 1983; Fairweather-Tait et al., 1983), an effect which further reduces protein ADC.

Dry-heating under pressure significantly reduced protein ADC in comparison with the value we found for raw chickpeas, despite the fact that this processing reduced trypsin inhibitor activity (TIA) by 30% and reduced tannins by 47% (Nestares et al., 1993a). The low digestive utilization of chickpea protein after this dry-heating was due to denaturation of the protein molecules (Bender, 1978), which reduced the bioavailability of some amino acids (Kirk, 1984). Soaking in solutions of different pH did not improve the ADC in comparison with that for raw chickpeas. Although the intake of soaked chickpeas was greater, so was fecal excretion of nitrogen, probably because soaking only partially reduced, but did not entirely remove, trypsin inhibitors or tannins (Nestares et al., 1993a). Soaking followed by cooking led to higher ADC than any other type of processing; this result was due the fact that increased protein intake was not accompanied by a further increase in fecal nitrogen excretion, probably because cooking removed trypsin inhibitors and partly reduced tannin content (Nestares et al., 1993a). In addition, cooking (in contrast to dry-heating) did not degrade proteins, probably because of the protective effect of the cooking water (Varela et al., 1967) and the lower cooking temperature and gentler cooking conditions we used. Our results are similar to those of Savage and Thompson (1993), who found that cooking in water improved the ADC of chickpeas and explained this effect as a result of the removal of trypsin inhibitors.

The metabolic utilization of chickpea protein, assessed as nitrogen balance, was lower than expected in view of the chemical score we obtained. Our analytical methods did not take into account amino acid imbalances or differences in their rates of absorption, nor did they account for a number of other factors such as the possible interactions between protein and other components of the diet.

The metabolic utilization of chickpea protein we report here is higher than the value we obtained under similar experimental conditions for the legume lentil (*Lens culinaris*) (Urbano et al., 1995). In contrast with chickpea, the chemical score of lentil protein showed that this legume is unsuitable for satisfying the requirements of growing rats for essential amino acids.

In the present study we found that nitrogen balance was lower than that reported by other authors for chickpea (Chavan et al., 1989; Combe et al., 1991; Savage and Thompson, 1993). The discrepancies are logical, in view of the fact that in our experiments chickpeas were the only source of food.

Basic-soaking (SB) and cooking preceded by soaking in any of the three media (SC, SAC, and SBC) significantly improved the metabolic utilization of protein. This effect was not necessarily associated with increased body weight; for example, in groups SC and SAC, body weight increased much less than we would have expected from the value we obtained for nitrogen retention. In groups SB and SBC, body weight increased mainly as a result of the changes in carbohydrates during basic-soaking and cooking. The utilization of carbohydrates probably increased since the digestibility of starch is affected by some processing and cooking technologies (Holm et al., 1988). Soaking in basic medium makes available an initially indigestible fraction of starch (retrograde amylose) and, thus, increases the nutritive utilization of starch (Tovar et al., 1990). This would account for the greater weight gain in animals fed with basic-soaked chickpeas than with chickpeas soaked in the other media. Similar findings were reported by Rao and Rao (1978), who tested a casein-methionine diet adjusted with chickpea starch and found that body growth was faster with cooked than with raw chickpeas, as a result of the increased availability of starch after cooking.

In conclusion, the chemical evaluation (aminogram, chemical score, crude protein, and protein nitrogen content) of protein from raw and processed chickpea showed that the high protein content and amino acid profile of this legume were adequate for satisfying the nutritional requirements of growing rats. All processes compared improved the palatability of chickpeas and increased food intake. The largest increases were found in rats fed with basic-soaked chickpeas and chickpeas cooked after soaking in distilled water or basic or acid medium. These findings may have been due to the removal or reduction of antinutritional factors (i.e., α -galactosides and tannins) during soaking and cooking. The improved nutritive utilization of chickpea protein with the basic-soaked diet and chickpeas cooked after soaking in distilled water or basic or acid medium was not related to increases in body weight; rather, weight gain was more closely related to modifications in carbohydrates caused by different processing methods.

ACKNOWLEDGMENT

We thank Ms. Karen Shashok for translating the original manuscript into English.

LITERATURE CITED

- Anderson, G. H.; Glanville, N. T.; Li, E. T. S. Amino acids and the regulation of quantitative and qualitative aspects of food intake. In *Amino Acids: Metabolism and Medical Applications*; Blackburn, G. L., Young, V. R. Y., Grant, J. P., Eds.; J. Wright-PSG Inc.: Littleton, MA, 1982.
- Bender, A. E. *Food Processing and Nutrition*; Academic Press: London, 1978; pp 219–254.
- Chavan, J. K.; Kadam, S. S., Salunkhe, D. K. Chickpea. In Handbook of World Food Legumes: Nutritional Chemistry,

Processing Technology, and Utilization; Salunkhe, D. K., Kadam, S. S., Eds.; CRC Press: Boca Raton, FL, 1989; Vol. I, pp 247–288.

- Combe, E.; Achi, T., Pion, R. Compared metabolic and digestive utilisations of faba bean, lentil and chickpea. *Reprod., Nutr., Dev.* **1991**, *31* (6), 631–646.
- Fairweather-Tait, S. J.; Gee, J. M.; Johnson, I. T. The influence of cooked kidney beans (*Phaseolus vulgaris*) on intestinal turnover and faecal nitrogen excretion in rats. *Br. J. Nutr.* **1983**, 49, 303–312.
- FAO/WHO. Protein Requirements. *Meeting on Nutrition and Advances*; FAO: Rome, 1966; No. 37.
- FAO/WHO. Energy and Protein Requirements, Report of a Joint FAO/WHO Ad Hoc Expert Committee. Rep. Ser. No 522, WHO: Geneva, 1973; p 63.
- Fernández, M. M.; Aranda, P.; López-Jurado, M.; Urbano, G.; Estrella, E.; Diaz, C.; Frias, J.; Prodanov, M.; Vidal-Valverde, C. Effect of processing on some antinutritive factors of faba beans: influence on protein digestibility and food intake in rats. In *Recent Advances of Research in Antinutritional Factors in Legume Seeds*; Van Der Poel, A. F. B., Huisman, J. H., Saini, S., Eds.; Wageningen Press: Wageningen, The Netherlands, 1993; pp 467–471.
- Geervani, P.; Theophilus, F. Effect of home processing on nutrient composition of certain high yielding legume varieties *Indian J. Nutr. Diet.* **1980**, *17*, 443.
- Harper, A. E. Effect of dietary protein content and amino acid pattern on food intake and preference. In *Handbook of Physiology. Alimentary Canal*; Code, C. F., Ed., American Physiological Society: Washington, DC, 1967; Vol. I, pp 399-410.
- Holm, J.; Lundquist, I.; Björck, I.; Eliasson, A.-N.; Asp, N. G. Degree of starch gelatinization, digestion rate of starch in vitro, and metabolic response in rats. *Am. J. Clin. Nutr.* **1988**, 47, 1010–1016.
- Johnson, D. J.; Anderson, G. H. The prediction of plasma amino acid concentration from diet amino acid content. *Physiol. Rev.* **1982**, 243, 99–103.
- Kirk, J. R. Biological availability of nutrients in processed foods. J. Chem. Educ. 1984, 61 (4), 364.
- Le Magnen, G. Body energy balance and food intake: a neuroendocrine regulatory mechanism. *Physiol. Rev.* **1983**, *63*, 314–386.
- López-Frias, M.; Llopis, J.; Montellano, M. A.; Urbano, G. Influence of gestation and hydrocortisone-acetate treatment on the nutritive utilization of protein. *Nahrung* **1985**, *29* (1), 11–18.
- Mole, S. Polyphenolics and the nutritional ecology of hervibores. In *Toxicants of Plant Origen, Phenolic*; Cheeke, P. R., Ed.; CRC Press: Boca Raton, FL, 1989; Vol. 4, pp 191–223.
- National Research Council. Nutrient Requirements of Laboratory Animals, 4th revised ed., National Academy of Sciences: Washington, DC, 1990.
- Nestares, T.; Barrionuevo, M.; Lopez-Frias, M.; Urbano, G.; Prodanov, M.; Frias, J.; Estrella, E.; Vidal-Valverde, C. Effect of processing on some antinutritive factors of chickpea: Influence on protein digestibility and food intake in rats. In *Recent Advances of Research in Antinutritional Factors in Legume Seeds*; Van Der Poel, A. F. B., Huisman, J. H., Saini, S., Eds.; Wageningen Press: Wageningen, The Netherlands, 1993a; pp 487–491.
- Nestares, T.; López-Jurado, M.; Sanz, A.; López-Frias, M. Nutritional Assessment of two vegetable protein concentrates in growing rats. *J. Agric. Food Chem.* **1993b**, *41*, 1282–1286.
- Peters, J. C.; Harper, A. E. Adaptation of rats to diets containing different levels of protein: Effects on food intake, plasma and brain amino acid concentrations and brain neurotransmitter metabolism. J. Nutr. 1985, 115, 382–398.
- Rao, C. N.; Rao, B. S. N. Influence of starches from different sources on protein utilization in rats. *Br. J. Nutr.* **1978**, *40*, 1–9.
- Rossi, M.; Germondari, I.; Casin, P. Comparison of chickpea cultivars: Chemical composition, nutritional evaluation, and oligosaccharide contents. *J. Agric. Food Chem.* **1984**, *32*, 811–814.

- Sanoja, S.; Bender, E. The effect of cooked legumes on mucosal cell turnover in the rat. *J. Nutr.* **1983**, *43*, 89.
- Santidrian, S. Consideration on protein nutritional aspects. *OFFARM* **1987**, *5*, 47–54.
- Savage, G. P.; Thompson, D. R. Effect of processing on the trypsin inhibitor content and nutritive value of chickpeas (*Cicer arietinum*). In *Recent Advances of Research in Anti-nutritional Factors in Legume Seeds*; Van Der Poel, A. F. B., Huisman, J. H., Saini, S., Eds.; Wageningen Press: Wageningen, The Netherlands, 1993; pp 435–440.
- Savitri, A.; Desikachar, H. S. R. A comparative study of flatus production in relation to the oligosaccharides content of some legumes. *Nutr. Rep. Int.* **1985**, *31*, 337–344.
- Singh, U.; Jambunathan, R. Relationship between non-protein nitrogen and total nitrogen in chickpea (*Cicer arietinum L.*) seed. J. Agric. Food Chem. **1981a**, 29, 423–425.
- Singh, U.; Jambunathan, R. Studies on *desi* and *kabuli* chickpea (*Cicer arietinum L.*) cultivars: Levels of protease inhibitors, level of polyphenolic compounds and *in vitro* protein digestibility. *J. Food Sci.* **1981b**, *46*, 1363–1367.
- Singh, U.; Jambunathan, R. Distribution of seed protein fractions and amino acid in different anatomical parts of chickpea (*Cicer arietinum* L.) and pigeon pea (*Canajus cajan* L.). *Qual. Plant-Plant Foods Hum. Nutr.* **1982**, *31*, 347-354.
- Singh, U.; Kherdekar, M. S.; Jambunathan, R. Studies on *desi* and *kabuli* chickpea cultivars: Levels of amylase inhibitors, levels of oligosaccharides and *in vitro* starch digestibility. *J. Food Sci.* **1982**, *47*, 510–512.
- Singh, U.; Kumar, J.; Gowda, C. L. L. The protein content of chickpea (*Cicer arietinum L.*) grown at different locations *Qual. Plant.*—*Plant Foods Hum. Nutr.* **1983**, *32*, 179.
- Stucki, V. P.; Harper, A. E. Effects of altering the ratio of indispensable to dispensable amino acids in diets for rats. *J. Nutr.* **1962**, *78*, 278–286.
- Tackman, J. M.; Tews; J. K.; Harper, A. E. Dietary disproportions of amino acid in the rats: effects on food intake, plasma and brain amino acids and brain serotonin. *J. Nutr.* **1990**, *120*, 521–533.
- Tews, J. K.; Good, S. S.; Harper, A. E. Transport of threonine and tryptophan by rat brain slices: Relation to other amino acids at concentrations found in plasma. *J. Neurochem.* **1978**, *31*, 581–589.
- Tovar, J.; Björck, I. M.; Asp, N.-G. Analytical and nutritional implications of limited enzymic availability of starch in cooked red kidney beans. J. Agric. Food Chem. 1990, 38, 488–493.
- Urbano, G.; López-Jurado, M.; Hernández, J.; Fernández, M.; Moreu, M. C.; Frias, J.; Diaz-Pollán, C.; Prodanov, M.; Vidal-Valverde, C. Nutritional assessment of raw, heated and germinated lentils. *J. Agric. Food Chem.* **1995**, *43*, 1871– 1877.
- Varela, G.; Moreiras, O.; Vidal, C.; Murillo, A.; Luque, J. A. Influencia del tratamiento térmico sobre la calidad de la proteína del cachuete. II. Acción protectora de la humedad y de la grasa y efecto de la glucosa y de la sacarosa (Influence of heat treatment on protein quality in the ground nut II. Protective action of humidity and fat, and effect of glucose and saccharose). *Proc. Nutr. Soc.* **1967**, *27*, 1–7.
- Varela, G.; Moreiras, O.; Carbajal, A.; Campo, M. National Study in Nutrition and Alimentation 1990/91; Publicaciones del Instituto Nacional de Estadística: Madrid, 1995.
- Vidal-Valverde, C.; Frias, J.; Valverde, S. Changes in the carbohydrate composition of legume after soaking and cooking *J. Am. Diet. Assoc.* **1993**, *93*, 547–550.
- Vidal-Valverde, C.; Frias, J.; Estrella, I.; Gorospe, M. J.; Ruiz, R.; Bacon, J. Effect of processing on some antinutritive factors of lentils *J. Agric. Food Chem.* **1994**, *42*, 2291–2295.

Received for review August 11, 1995. Accepted May 28, 1996.[®] This study was supported by Projects CICYT ALI88-466-C02-02 and CICYT ALI 91-1092-C02-01.

JF950545Q

 $^{\otimes}$ Abstract published in Advance ACS Abstracts, July 15, 1996.