

# Effect of Processing Methods on the Calcium, Phosphorus, and Phytic Acid Contents and Nutritive Utilization of Chickpea (*Cicer arietinum* L.)

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The effect of chickpea (*Cicer arietinum* L.) processing methods on the nutritive utilization of calcium and phosphorus and on phytic acid, a seed component that affects mineral utilization, was studied. Chemical and biological methods were used for nutritional determinations in growing rats. The digestive utilization of calcium from raw chickpea was adequate for growing rats; however, processing resulted in a slight decrease. The metabolic utilization of chickpea calcium was low because of the low rates of net absorption. This was reflected in the decreased calcium levels in longissimus dorsi muscle in the absence of mobilization of calcium from the femur. Soaking in acid solution followed by cooking decreased phytic acid content, suggesting that processing made part of the phytic acid phosphorus available. The absorbed phosphorus was greater than the nonphytic phosphorus supplied by the diet. The digestive utilization of phosphorus was similar in processed and raw chickpeas, despite the loss of soluble anion as a result of processing. These results may indicate the contribution of phosphorus in the form of inositol hexaphosphate–phosphorus.

**Keywords:** Chickpeas; calcium; phosphorus; phytic acid; processing techniques; nutritive utilization

## INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the fifth most widely grown legume in the world (Chavan et al., 1989) and is the most widely consumed legume in Spain (Varela et al., 1995). Chickpea crops improve the physical and biological properties of the soil. This legume also fixes atmospheric nitrogen and thus requires less chemical fertilization, a characteristic with obvious ecological advantages.

In addition to being an important source of protein, chickpea is also reported to be a good source of minerals (Jambunathan and Singh, 1981; Attia et al., 1994; Nestares et al., 1997). This legume supplies larger amounts of calcium and phosphorus than do other legumes (Chavan et al., 1989) and contains more calcium than whole cow's milk (120 mg/100 g). The amount of phosphorus in chickpeas approaches that found in the major sources of this element in the Mediterranean diet (Mataix et al., 1995).

However, its use as a food is limited by antinutritional factors that reduce the nutritive value of this legume. For human consumption, chickpeas must be processed to improve mineral composition and bioavailability. The effects of processing vary notably depending on the techniques and experimental conditions (Singh, 1985; Vidal-Valverde and Frías, 1991; Nestares et al., 1996, 1997). Processing also affects factors such as protein, fiber, and phytate content, which in turn can enhance or reduce the bioavailability of minerals (Kumar et al., 1978; Rao and Deosthale, 1982; Jood et al., 1987; Duhan et al., 1989; Torre et al., 1991; Nestares et al., 1997).

These effects make it difficult to draw conclusions about the influence of processing on mineral composition and bioavailability in chickpeas; consequently, each set of experimental conditions needs to be studied separately. We report the effects of different widely used processing techniques on the composition and nutritive utilization of calcium and phosphorus in chickpeas. We used these techniques to remove antinutritional factors, to improve the palatability and nutritive utilization of this legume (Nestares et al., 1996), and to reproduce processing methods that are commonly used in the home and in industrial plants to obtain food products for babies and elderly persons.

## MATERIALS AND METHODS

**Samples.** Raw, dried chickpeas (R) (*C. arietinum* L. var. Lechosa) were grown in Andalusia (southern Spain). The seed lots were processed and divided into eight treatments: R, raw; 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 pressure at 120 °C and 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 (w/v). 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 (w/v). The cooking water was drained off, and the seeds were blended and lyophilized.

**Analytical Techniques.** Moisture content was determined by oven-drying at 105 ± 1 °C until a constant weight was obtained. Ash was measured in samples reduced to a constant

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weight at 500 °C. Inositol hexaphosphate content was determined according to the method of Latta and Eskin (1980). Mineral content (calcium and phosphorus) was determined in the diets, feces, femur, longissimus dorsi muscle (previously ashed and dissolved in 5 N HCl), and urine (untreated sample). Calcium concentrations were determined with a Perkin-Elmer 1100 B atomic absorption spectrophotometer. Inorganic phosphorus was determined by visible spectrophotometry with the colorimetric technique of Fiske and Subbarow (1925).

**Biological Methods.** *Experimental Design and Diet.* We used a biological balance technique. We recorded food intake and changes in body weight and then calculated calcium and phosphorus intake and fecal and urinary calcium and phosphorus excretion. Eight experiments of 10 days each were done in which raw or processed chickpeas as above were the only source of food. 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 analysis.

In each experiment we used 10 young albino Wistar rats (5 males, 5 females). The growing animals (recently weaned), with an initial body weight of  $58.8 \pm 1.5$  g, were housed in individual metabolic cages kept in a thermoregulated room ( $22 \pm 1$  °C) with a controlled 12 h light/dark period (lights on at 9:00 a.m.). The rats were handled at all times in accordance with current European regulations regarding laboratory animals.

*Biological Indices.* The following parameters were determined for each group, according to the formulas given below: apparent digestibility coefficient (ADC) (1) for calcium and phosphorus; calcium and phosphorus retention (balance) (2); and percent calcium or phosphorus retention/calcium or phosphorus absorption (%R/A) (3):

$$\text{ADC} = [(I - F)/I] \times 100 \quad (1)$$

$$\text{balance} = I - (F + U) \quad (2)$$

$$\%R/A = \{[I - (F + U)](I - F)\} \times 100 \quad (3)$$

In accordance with the formulas recommended by the National Research Council (1990), the factors used were *I* (mineral intake), *F* (fecal mineral), and *U* (urinary mineral). Calcium and phosphorus intakes were expressed as milligrams per rat per day.

**Statistical Methods.** The results from all of the experiments and analyses were expressed as the mean values  $\pm$  SEM. Data were tested statistically by analysis of variance using Statgraphic Statistical Graphics 5.0 System software (1991) (Statistical Graphics Corp., Rockville, MD) with an IBM Personal System/2 Model 20 computer. Differences were considered significant when  $P < 0.05$ .

## RESULTS

**Chemical Analysis.** Table 1 gives the values for ash, calcium, phosphorus, and inositol hexaphosphate (IHP) content in raw and processed chickpea diets. Raw chickpeas contained 1.37 g of calcium/kg of sample. Soaking alone or followed by cooking decreased calcium by 10–25%, with greater reductions after SAC than after SC. As expected, heating had no effect on calcium content.

Raw chickpeas contained 3.02 g of total phosphorus/kg of sample. Processes S, SA, SC, SAC, and SBC reduced soluble phosphorus content by 10–15%. However, treatments SB and H had no significant effect on this variable.

Of the total phosphorus in raw chickpeas, IHP accounted for 5.9 mg/g (55.8%). Processing had no significant effect on IHP content, with the exception of

**Table 1. Ash, Calcium, Phosphorus, and Phytic Acid Composition (in Dry Matter) of Raw and Processed Chickpeas**

| diet <sup>a</sup> | ash <sup>b</sup><br>(%) | Ca <sup>b</sup><br>(mg/100 g of diet) | P <sup>b</sup><br>(mg/100 g of diet) | phytic acid <sup>b</sup><br>(mg/g of diet) |
|-------------------|-------------------------|---------------------------------------|--------------------------------------|--|
| R                 | 28.79                   | 1.37                                  | 3.02                                 | 5.9  |
| H                 | 29.09                   | 1.35                                  | 3.06                                 | 6.3  |
| S                 | 27.67                   | 1.18                                  | 2.75                                 | 5.1  |
| SA                | 26.26                   | 1.05                                  | 2.74                                 | 5.8  |
| SB                | 26.90                   | 1.15                                  | 3.00                                 | 5.2  |
| SC                | 29.70                   | 1.26                                  | 2.68                                 | 5.3  |
| SAC               | 19.10                   | 1.01                                  | 2.59                                 | 4.8  |
| SBC               | 19.60                   | 1.04                                  | 2.76                                 | 5.8  |

<sup>a</sup> Abbreviations: 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; SBC, chickpeas soaked in basic medium and cooked. <sup>b</sup> Standard errors ranged from  $\pm 0.01$  to  $\pm 0.001$ .

treatment SAC, which reduced IHP by 20% to 4.8 mg/g, that is, 45% of the total phosphorus content.

**Biological Analysis.** Calcium intake, expressed as grams of diet per 100 g of body weight, was significantly greater in all of the processed diets than in the raw sample. The greatest increases were found in groups SB, SC, and SAC (Nestares et al., 1996).

The digestive utilization of calcium, calculated as the ADC (Table 2), was 83.5% in raw chickpeas. All types of processing significantly reduced the ADC, with the exception of treatment S. The greatest decreases were observed after SAC and SBC.

Calcium retention (Table 2) was generally low and was significantly decreased in groups SA, SAC, and SBC in comparison with animals that ate raw chickpeas. Soaking in a basic solution (SB) led to a significantly better metabolic balance than in the other groups.

The metabolic utilization of calcium, assessed as the ratio of retained to absorbed calcium (Table 2), decreased significantly in all treatments except SB, which led to significantly higher values than in all other groups.

We found no significant differences in femur or muscle weight between animals fed with raw or processed chickpeas. Calcium content in bone and muscle (expressed as grams of ash) likewise did not differ significantly between groups of animals (Table 3), except for muscle in group SA and for femur in group H, both of which gave values higher than in other groups.

Animals fed with diet R, SA, or SAC had significantly lower total phosphorus intakes than the other groups (Table 4). This intake was significantly higher in groups S, SC, and SCB than after other treatments. Phosphorus intakes in groups H and SB were significantly higher than in the other groups.

Phosphorus absorption in absolute terms (Table 4) paralleled total phosphorus intake. Soaking in distilled water or basic solution, with or without cooking, improved absorption (Table 4, groups S, SB, SC, and SCB). This increase was significant in comparison with groups SA and SAC. Cooking after soaking in water or acid solution had no effect on phosphorus absorption in comparison with soaking alone. However, cooking after soaking in basic solution reduced the beneficial effects of soaking alone in basic solution.

The digestive utilization of phosphorus, assessed as the ADC, was significantly lower in group SA than in all other groups. Treatment SB significantly increased the ADC in comparison with no treatment and soaking

**Table 2. Digestive and Metabolic Utilization of Calcium**

| diet <sup>a</sup> | Ca intake (mg/rat/day)    | apparent digestibility coefficient | balance                   | Ca retention/<br>Ca absorption (%) |
|-------------------|---------------------------|------------------------------------|---------------------------|------------------------------------|
| R                 | 8.18 ± 0.30               | 83.46 ± 2.92                       | 5.66 ± 0.23               | 83.36 ± 1.30                       |
| H                 | 9.72 ± 0.31 <sup>a</sup>  | 77.20 ± 3.94 <sup>b</sup>          | 5.91 ± 8.45 <sup>a</sup>  | 77.92 ± 1.69 <sup>b</sup>          |
| S                 | 8.89 ± 0.31 <sup>ab</sup> | 82.57 ± 1.46                       | 5.69 ± 0.19 <sup>ab</sup> | 77.77 ± 0.84 <sup>bc</sup>         |
| SA                | 6.61 ± 0.11 <sup>c</sup>  | 78.36 ± 1.25 <sup>bc</sup>         | 3.81 ± 0.13 <sup>c</sup>  | 73.47 ± 0.64                       |
| SB                | 10.27 ± 0.20 <sup>a</sup> | 79.67 ± 1.64 <sup>bcd</sup>        | 7.06 ± 0.24               | 86.16 ± 1.05 <sup>d</sup>          |
| SC                | 9.10 ± 0.42 <sup>ab</sup> | 77.81 ± 1.84 <sup>bcd</sup>        | 5.48 ± 0.47 <sup>ab</sup> | 76.40 ± 2.41 <sup>bc</sup>         |
| SAC               | 7.09 ± 0.13 <sup>cd</sup> | 72.62 ± 1.08 <sup>e</sup>          | 3.42 ± 0.14 <sup>cd</sup> | 66.29 ± 1.69                       |
| SBC               | 7.47 ± 0.23 <sup>cd</sup> | 70.63 ± 0.94 <sup>e</sup>          | 3.79 ± 0.19 <sup>cd</sup> | 71.64 ± 2.09 <sup>d</sup>          |

<sup>a</sup> Abbreviations: see footnote *a* of Table 1.

**Table 3. Calcium and Phosphorus Content in Bone (Femur) and Muscle (Longissimus Dorsi)**

| diet <sup>a</sup> | muscle                      |                              |                             | bone                        |                              |                             |
|-------------------|-----------------------------|------------------------------|-----------------------------|-----------------------------|------------------------------|-----------------------------|
|                   | mg of ash/g                 | mg of Ca/g of ash            | mg of P/g of ash            | mg of ash/g                 | mg of Ca/g of ash            | mg of P/g of ash            |
| R                 | 56.74 ± 0.52 <sup>a</sup>   | 6.50 ± 1.08 <sup>a</sup>     | 157.0 ± 2.50 <sup>a</sup>   | 390.8 ± 26.3                | 372.2 ± 11.2 <sup>a</sup>    | 151.44 ± 4.9 <sup>a</sup>   |
| H                 | 57.96 ± 1.07 <sup>a</sup>   | 13.81 ± 2.40 <sup>b</sup>    | 160.6 ± 2.83 <sup>a</sup>   | 212.5 ± 16.1 <sup>a</sup>   | 512.4 ± 54.8                 | 194.58 ± 20.1 <sup>b</sup>  |
| S                 | 52.45 ± 1.11 <sup>b</sup>   | 9.72 ± 1.76 <sup>abc</sup>   | 170.0 ± 1.29 <sup>b</sup>   | 235.7 ± 8.0 <sup>a</sup>    | 401.3 ± 4.8 <sup>ab</sup>    | 177.29 ± 2.0 <sup>bc</sup>  |
| SA                | 54.25 ± 1.62 <sup>abc</sup> | 30.86 ± 3.57                 | 177.7 ± 4.05 <sup>c</sup>   | 177.5 ± 6.9 <sup>b</sup>    | 393.9 ± 7.7 <sup>abcd</sup>  | 186.38 ± 3.1 <sup>bcd</sup> |
| SB                | 55.16 ± 2.06 <sup>acd</sup> | 8.95 ± 1.15 <sup>abcd</sup>  | 171.7 ± 5.34 <sup>bcd</sup> | 199.1 ± 10.2 <sup>abc</sup> | 358.9 ± 12.1 <sup>abcd</sup> | 183.8 ± 3.2 <sup>bcd</sup>  |
| SC                | 63.28 ± 0.43                | 8.99 ± 2.06 <sup>abcde</sup> | 102.1 ± 2.71                | 190.1 ± 6.6 <sup>abc</sup>  | 358.6 ± 3.3 <sup>abcde</sup> | 200.4 ± 14.2 <sup>bcd</sup> |
| SAC               | 53.71 ± 0.95 <sup>bcd</sup> | 12.94 ± 5.00 <sup>bcd</sup>  | 185.1 ± 3.31                | 145.8 ± 4.31 <sup>d</sup>   | 338.9 ± 7.2 <sup>adef</sup>  | 180.6 ± 2.3 <sup>bcd</sup>  |
| SBC               | 49.76 ± 1.05                | 7.53 ± 1.31 <sup>acde</sup>  | 169.1 ± 2.40 <sup>bd</sup>  | 160.4 ± 10.7 <sup>bd</sup>  | 331.2 ± 18.2 <sup>adef</sup> | 165.7 ± 8.9 <sup>acef</sup> |

<sup>a</sup> Abbreviations: see footnote *a* of Table 1.

**Table 4. Digestive and Metabolic Utilization of Phosphorus**

| diet <sup>a</sup> | P intake (mg/rat/day)      | apparent digestibility coefficient | balance                    | P retention/<br>P absorption (%) |
|-------------------|----------------------------|------------------------------------|----------------------------|----------------------------------|
| R                 | 17.85 ± 0.65 <sup>a</sup>  | 78.41 ± 1.09 <sup>a</sup>          | 6.41 ± 0.52 <sup>a</sup>   | 45.33 ± 2.82 <sup>a</sup>        |
| H                 | 22.91 ± 0.74               | 79.71 ± 1.09 <sup>ab</sup>         | 8.09 ± 0.60 <sup>b</sup>   | 43.82 ± 1.93 <sup>ab</sup>       |
| S                 | 20.85 ± 0.72 <sup>b</sup>  | 80.03 ± 1.93 <sup>abc</sup>        | 3.50 ± 0.28 <sup>c</sup>   | 21.48 ± 2.09 <sup>c</sup>        |
| SA                | 17.27 ± 0.29 <sup>ac</sup> | 73.83 ± 1.58                       | 2.97 ± 0.48 <sup>c</sup>   | 22.95 ± 3.37 <sup>c</sup>        |
| SB                | 26.89 ± 0.52               | 83.07 ± 1.02 <sup>d</sup>          | 11.30 ± 0.67               | 50.50 ± 2.59 <sup>ad</sup>       |
| SC                | 19.59 ± 0.77 <sup>bd</sup> | 83.71 ± 1.49 <sup>de</sup>         | 7.50 ± 0.99 <sup>abd</sup> | 44.39 ± 4.32 <sup>abe</sup>      |
| SAC               | 18.12 ± 0.35 <sup>ac</sup> | 81.19 ± 0.82 <sup>bcd</sup>        | 6.52 ± 0.31 <sup>ad</sup>  | 44.28 ± 1.79 <sup>abe</sup>      |
| SBC               | 19.81 ± 0.62 <sup>bd</sup> | 81.61 ± 1.01 <sup>bcd</sup>        | 8.64 ± 0.52 <sup>bd</sup>  | 53.26 ± 2.32 <sup>d</sup>        |

<sup>a</sup> Abbreviations: see footnote *a* of Table 1.

in distilled water or acid solution, despite the fact that group SB had the highest total phosphorus intake. In general, soaking in basic solution and soaking before cooking (regardless of the type of solution used) improved the digestive utilization of total phosphorus.

Total phosphorus retention (Table 4) was significantly reduced by soaking in water (S) or acid solution (SA). However, soaking followed by cooking (SC and SAC) significantly improved total phosphorus retention to levels similar to that found in raw chickpeas. Soaking in basic solution improved phosphorus retention, but cooking (SBC) reduced retention, although the final value was still higher than in unprocessed chickpeas. Heating without prior soaking (H) also improved phosphorus retention in comparison with no treatment.

The ratio of total phosphorus retained to total phosphorus absorbed (Table 4) showed that soaking in distilled water (S) or acid solution (SA) significantly reduced phosphorus retention, because of the increase in urinary excretion of this element. When these treatments were followed by cooking (SC and SAC), retention improved, attaining values close to that found for raw chickpeas. Soaking in basic solution, with or without subsequent cooking, significantly improved the %R/A ratio.

The lowest phosphorus content in ashed bone was found in group SBC, and the lowest value in ashed muscle, in group SC. We found no significant differences in any of the other groups.

## DISCUSSION

**Chemical Analysis of Diets.** The mineral and ash contents of the raw chickpeas analyzed were within the range reported in earlier studies (Chavan et al., 1989; Attia et al., 1994), that is, between 2.04 and 4.67 g/100 g of dry matter. The ash content was significantly lower in diets SAC (33.7%) and SBC (31.9%) than in raw chickpeas. Decreases in mineral content after cooking were reported earlier (Meiners et al., 1976; Kumar et al., 1978; Attia et al., 1994), although in the present study, the decreases were slightly smaller than those found by other authors.

The contents of calcium and phosphorus in raw chickpeas were within the range found for these mineral (Meiners et al., 1976; Jambunathan and Singh, 1981; Attia et al., 1994). Processing reduced the calcium content by ~10–25%; a similar reduction (20%) after cooking was reported by Attia et al. (1994). Soaking, with or without subsequent cooking, reduced phosphorus content by 10–15%, that is, by somewhat more than the 8.5% reduction found by Attia et al. (1994) after cooking. Soaking in basic solution did not significantly affect phosphorus content. The differences in the reductions in mineral content after processing may be due to differences in the experimental conditions used here and in earlier studies and to the use of different varieties of *C. arietinum*.

Of the total amount of phosphorus in raw chickpeas, 55.8% consisted of IHP. Although the technique we used

to detect phytic acid in the diet (Latta and Eskin, 1980) does not measure this compound specifically, IHP accounts for 90% of the total phytic acid content in legumes (Sandberg and Ahderinne, 1986; Lehrfeld, 1989; Lehrfeld and Morris, 1992). The amount of phytic acid in our samples (5.9 mg/g of dry matter) is lower than the figures reported by Duhan et al. (1989) and Khan et al. (1988) in several different varieties of chickpea (between 7.48 and 12.2 mg/g of dry matter); however, our values are higher than those reported by Kumar et al. (1978). The disparities between the phytic acid contents in chickpea found by different authors may be due to differences in analytical techniques and to the large differences between varieties, edaphological and climatological conditions, and seed development (Spiller, 1986).

None of our treatments significantly modified the phytic acid content in chickpeas with the exception of SAC, which led to a decrease of ~20%, an effect similar to that reported in *Vicia faba* (Fernandez et al., 1997). This treatment also led to the greatest decrease in total phosphorus. Soaking in acid solution followed by cooking may have allowed endogenous phytase to act (acid pH and 50 °C) (Lolas and Markakis, 1977; Eskin and Wiebe, 1983). The enzyme may later have been destroyed when the temperature rose to 100 °C, and this may account for the low rate of transformation of phytic acid in diet SAC.

**Biological Analysis of Calcium.** We found a high ADC for chickpea calcium (83.46%); the figure was close to that reported for a casein–methionine diet (86.76 ± 3.83%) (Urbano et al., 1999). Therefore, net absorption of calcium from raw chickpeas was adequate for our experimental animals. The lower fecal excretion of calcium was responsible for the high ADC of chickpea calcium.

Processing modified some of the nutrient components that can affect calcium absorption. Under our experimental conditions, dietary protein had no effect on calcium utilization, as shown by the similarity in the ADC values found in rats fed a chickpea diet and in animals given a casein-supplemented diet (Urbano et al., 1999). According to Khan and Chakrabarti (1978), the protein content in the diets tested here ensured an adequate digestive utilization of calcium. Heating under pressure (diet H) denatures protein; this may account for the low digestive utilization of calcium found in this group. However, the other treatments, despite improving the digestive utilization of protein (Nestares et al., 1996), decreased the digestive utilization of calcium slightly.

Soaking alone or followed by cooking increases fiber (Vidal-Valverde and Frías, 1991; Vidal-Valverde et al., 1993), that is, cellulose (Nestares et al., 1997), which may have complexed with the calcium and thus trapped the mineral on the surface of the cellulose molecule (Torre et al., 1991). This may explain the lower digestive utilization of calcium in these groups than in rats fed with raw chickpeas. The formation of these complexes was not significantly affected by pH (Torre et al., 1992). Our findings thus confirm the hypothesis proposed by Spiller (1986) that calcium absorption decreases as a result of dietary enrichment with fiber. Although our results showed no correlation between calcium intake and absorption, the different soaking and cooking conditions may have led to losses of different amounts of soluble calcium; this may account for variations in the

digestive and metabolic utilization of the mineral, as described by Kaup et al. (1990).

Despite the increase in fiber and loss of calcium due to solubilization during processing, the ADC for calcium in processed chickpeas was similar to that found for a standard casein–methionine diet (Urbano et al., 1999).

The metabolic utilization of chickpea calcium as a function of calcium balance was low because of the rates of urinary excretion and net absorption of the cation (Table 2). However, we found a high correlation ( $r = 0.9708$ ,  $P = 0.000$ ) between calcium absorption and retention, which indicates that calcium levels were regulated in the digestive tract. High concentrations of parathyroid hormone (as a result of the low calcium intake) probably increased calcium retention in a manner directly proportional to calcium absorption.

Our experimental conditions (diets that did not totally satisfy the nutritional requirements for calcium) affected muscle tissue, that is, the compartment involved in maintaining plasma levels of the cation within physiological limits (Van Berestejin et al., 1993). However, femoral calcium remained within levels considered normal for the growing rats. These results may have been due to the relatively short experimental period (10 days) used in the present study.

**Biological Analysis of Phosphorus.** The digestive utilization of chickpea phosphorus, assessed as the ADC, was nearly 95% in rats given a diet containing 20% casein–methionine, a value higher than that found in other legumes (Fernández et al., 1997).

The ADC of phosphorus was not improved by heating under pressure despite an increase of ~23% in total phosphorus absorption. This finding indicates that the increase in absorption reflected the 22% increase in intake, rather than an effect of processing.

In general, the ADC for phosphorus from processed chickpeas was similar to or higher than that from raw chickpeas despite the loss of soluble anion due to processing. This may reflect the contribution of phosphorus in the form of IHP, as in raw chickpeas, 55.8% of the total phosphorus content was in the form of this compound, which is not completely utilizable. During digestion, chickpea IHP is transformed into inorganic phosphorus and inositols, which contain fewer phosphate groups because of the action of phytase in the legume itself (Ranhotra and Loewe, 1975; Sandberg and Ahderinne, 1986; Yang et al., 1991; Jany, 1993) and endogenous phytase in the rat (Yang et al., 1991). These enzymes probably increase the amount of anion available from IHP in the intestinal lumen, and this form may have been absorbed together with phosphorus from nonphytic sources. This hypothesis is supported by our finding that the amount of phosphorus absorbed (probably as IHP) was greater than the amount of nonphytic phosphorus supplied by the diet.

Under our experimental conditions the low calcium content of the diet increased the availability of phosphorus in the form of IHP, by facilitating the action of phytase (Lei et al., 1994). Enzyme activity is more efficient when phytic acid is in the free form, that is, not bound to divalent metals (Nahapetian and Young, 1980).

Net phosphorus absorption was greatest in group SB because phosphorus intake was significantly higher than in other groups, and none of the element was lost because of solubilization. The effect of greater intake

on net absorption was also evident in rats fed with chickpeas heated under pressure (group H).

The homeostatic regulation of phosphorus metabolism is known to involve variations in food intake and renal excretion. Under our experimental conditions the high rate of urinary excretion (because of the low calcium retention) and the relatively low rate of phosphorus absorption led to a lower metabolic utilization of phosphorus.

Phosphorus retention was very low; despite the effects of different types of processing, we found no differences between groups in anion concentrations in femur or muscle. Similar results have been reported for *V. faba* (Fernández et al., 1997). The tissue levels of phosphorus, expressed as milligrams of P per gram of ash, were similar to those in growing rats fed a casein-methionine diet (Fernández et al., 1997), despite the fact that this diet increased phosphorus retention. This finding may reflect the slower growth rate in rats fed the chickpea diets and the short experimental period used here.

In conclusion, processing chickpeas removed antinutritional factors (for example, phytic acid, trypsin inhibitor activity,  $\alpha$ -galactosides, and tannins) that limit the consumption of this legume and affect its nutritive utilization. Processing did not affect the ADC or metabolic utilization (%R/A) of calcium; these values remained near those found when growing rats were fed a standard casein-methionine diet. The digestive utilization of phytic acid phosphorus was improved by processing. Of the processing techniques we compared, soaking in basic solution led to the greatest improvements in nutritive utilization. This finding is of interest, as soaking before cooking is a simple and cheap technique that can be used both in the home and by industries that produce food products for nutritionally vulnerable persons with high calcium and phosphorus requirements.

#### ACKNOWLEDGMENT

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

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Received for review July 29, 1998. Revised manuscript received March 5, 1999. Accepted March 23, 1999. This study was supported by Projects CICYT ALI88-466-C02-02 and CICYT ALI 91-1092-C02-01.

JF9808325