Effect of Soaking Prior to Cooking on the Levels of Phytate and Tannin of the Common Bean (Phaseolus vulgaris, L.) and the Protein Value

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Summary The effect of soaking in domestic processing, on the nutritive value of the common bean (Phaseolus vulgaris, L.) cv IAC-Carioca, was studied. Five treatments were carried out with experimental diets, and offered to male, recently weaned Wistar rats. The protein sources were, respectively, control diet (casein) (CC), casein plus the soluble solids found in the soaking water (CSS), freeze dried bean cooked without soaking (BNS), freeze dried bean cooked with the non-absorbed soaking water (BSW), freeze dried bean cooked without the non-absorbed soaking water (BSNW), and an aproteic diet (AP) for corrective purposes. The anti-nutritional factors (phytates and tannins), were determined in the differently processed beans and in the soaking water. The following values for the reduction of phytates were obtained: BNS (20.9%), BSNW (60.8%) and BSW (53.0%), and the tannins were reduced by: BNS (86.6%), BSNW (88.7%) and BSW (89.0%). No significant differences were observed between the various treatments using the common bean as protein source, with respect to the net protein ratio (NPR). With respect to the digestibility corrected by non-protein diet, values varying between 94.1% and 94.6% for casein, and between 57.5% and 61.4% for the common bean, were observed, the treatment BNS being more digestible. It was concluded that soaking did not interfere with the NPR of the experimental diets containing the common bean as protein source, nor did it reduce the tannin content. However soaking was capable of reducing the phytate levels in the common bean. On the other hand, soaking was unable to increase the protein digestibility of the common bean, since the treatment BNS showed the highest value for digestibility.

Key Words common bean, soaking, tannins, phytates, proteins, nutritive value

The common bean (Phaseolus vulgaris, L.), important component of the diet in all social classes, has thus acquired priority status amongst Brazilian agricultural products. It is cultivated by a wide range of agriculturists, on different scales, using different production systems and in different physical and socio-economic environments. Brazil is the largest world producer of beans, with an estimated annual production of three million tons, in an area of 4.1 million hectares (1). The elevated costs of producing protein of animal origin reflect in the use of other, cheaper protein sources, with the emphasis on the use of vegetables as a source of protein for human consumption. However, vegetable proteins are usually of lower quality than animal proteins, either as a result of lower digestibility, lack of essential amino acids or presence of substances which prejudice the bioavailability of proteins and minerals.

According to Khokhar and Chauhan (2), domestic processing and cooking methods are known to reduce the anti-nutritional factors, improving the nutritional value of legumes. Some authors have observed that the soaking of beans made their cooking easier, reducing the cooking time. Other methods (3), such as removal of the cortex and cooking, autoclaving and pressure cooking and germination, have been shown to be efficient in the reduction of the anti-nutritional factors of legumes. The question of whether to use soaking or not is controversial, although the evidence tends to indicate its use. A further controversy is the question of whether to discard the non-absorbed soaking water prior to cooking, or not. In practice, the use of both procedures can be observed, based purely on experience, with no scientific basis, such that more profound aspects, such as the existence of anti-nutritional factors and the relationship of these with the soaking process prior to cooking, have still not been well studied.

The present study aimed at evaluating the effect of soaking prior to cooking on the phytate and tannin contents of beans, and the effect of these levels on the growth of experimental animals and on protein digestibility, aiming at a better use of this legume in human feeding.

MATERIALS AND METHODS

Protein sources. The common bean (Phaseolus vulgaris, L.; cv IAC-Carioca, batch 09/99), was used, provided by the Centre for the Production of Propagated...
Material of The Campinas Agronomic Institute, SP, Brazil, the control being commercial casein (M. CASSAB Comércio e Indústria Ltda., SP). The beans were soaked at room temperature (25°C) for a period of 16 h in the proportion 3:1 (water:beans), and then cooked with the same proportion water:beans (3:1) in a domestic pressure (14.1 psi) cooker for 40 min after the air exhaustion (water steam flux continuous). The beans were then frozen, freeze-dried and ground (70 mesh), to give a dry flour.

Chemical characterization. The following compositional characteristics were determined in the raw and cooked beans, with and without soaking: moisture according to A.O.A.C. (4), crude protein by the semimicro Kjeldahl method (5), using a conversion factor of 5.4 for bean protein (6) and of 6.38 for casein, total lipids (7), ash (8), crude fibre (9) and carbohydrates by difference. The amino acid compositions of the processed bean cultivar and the casein used were determined according to the method of Spackman et al. (10) and Beckman Instruments (11). The triptophane was not determine, because it is destroyed in the acid hydrolysis. Phytate content was determined using the methodology proposed by Latta and Eskin (12). The methodology of Deshpande et al. (13) was used to determine the tannin contents. All the samples were adequately ground (70 mesh) and maintained under refrigeration until analysed. The analyses were carried out in triplicate, with 5 repetitions of each.

Animals and experimental diets. Forty eight recently weaned, male albino Wistar rats (21 d, 45-50 g), provided by the Animal Production Centre of the State University of Campinas, SP, Brazil, were maintained in individual cages for a period of 15 d. The animals received water and the diet “ad libitum”, the environment being maintained at a temperature of 23 ± 1°C and relative humidity of 50–60%, with a light/dark cycle of 12 h. The protocol of the Commission of Ethics in Animal Experimentation of the Biology Institute of UNICAMP, Campinas, SP, Brazil, was observed. The experimental diets were elaborated according to the composition of the diet AIN-93G, cited in Reeves et al. (14), with the exception of the protein content (12%) (15, 16) in order to compare the protein nutritive values. All the diets were completed with carbohydrates to make up 100% (17), such that they became iso-protein (12.0 ± 0.5%) and iso-energetic (350.9 ± 37.9 kcal/100 g). The diets energetic values were calculated considering the average caloric values usually utilized of 4.1 kcal/g for proteins and carbohydrates, and 9.3 kcal/g for lipids.

Biological assay. The assay lasted 15 d, 5 d being for adaptation and 10 d for the calculation of the net protein ratio (NPR) (16). The faeces of the animals were collected the first 5 d after adaptation, for a subsequent analysis of nitrogen and digestibility of the protein sources (17). All the nitrogen analyses were carried out in triplicate for each rat, also registering the weight gain and diet consumption during the period. The animals were submitted to the following diets: CC, casein control; CSS, casein + soluble solids; 7.50 g of the solu-

ble solids, containing 2.69 g phytate and 0.90 g tannin, were added; AP, aprotic diet; BNS, bean cooked without soaking; BSNW, bean cooked without the non-absorbed soaking water; BSW, bean cooked with the non-absorbed soaking water.

Statistical analysis. The results obtained in the biological assays and the chemical determinations were submitted to an analysis of variance (ANOVA) and to Tukey’s means test, using the programme STATISTICA 6.0®, Stat Soft (Tulsa, OK, USA), considering p<0.05 as the minimum acceptable probability for the difference between the means.

RESULTS AND DISCUSSION

Chemical composition of the raw material

Table 1 shows the proximate composition of the raw material used as protein source. The results found for the common bean are in agreement with those cited in the literature (18, 19). The amino acid compositions of the processed bean cultivar (BNS) and the casein used are shown in the Table 2, and the results found are also in agreement with those determined by Oliveira (20).

| Components (%) | RB  | BNS |
|----------------|-----|-----|
| Protein        | 17.0| 17.7|
| Lipid          | 2.0 | 2.5 |
| Crude fibre    | 4.4 | 6.3 |
| Ash            | 3.8 | 4.4 |
| Moisture content| 11.0| 3.1 |
| Carbohydrates* | 61.9| 66.7|
| Total solids   | 89.0| 96.9|

* Determined by difference. RB, raw bean. BNS, bean freeze-dried and cooked without soaking.

| Amino acid                  | BNS  | Casein |
|-----------------------------|------|--------|
| Aspartic acid               | 14.2 | 8.4    |
| Threonine                   | 5.9  | 5.5    |
| Serine                      | 6.3  | 7.1    |
| Glutamic acid               | 18.4 | 29.6   |
| Proline                     | 3.9  | 9.3    |
| Glycine                     | 5.3  | 2.2    |
| Alanine                     | 5.4  | 3.4    |
| Valine                      | 9.6  | 7.1    |
| Metionine                   | 1.0  | 2.9    |
| Sulphurated amino acids (Cys+Met) | 1.3  | 2.9    |
| Isoleucine                  | 5.7  | 5.2    |
| Leucine                     | 9.6  | 9.9    |
| Phenilalanine               | 6.6  | 5.7    |
| Aromatic amino acids (Tyr+Phe) | 9.1  | 11.2   |
| Histidine                   | 3.9  | 3.1    |
| Lysine                      | 8.1  | 7.9    |
| Arginine                    | 6.8  | 4.2    |

BNS, common bean freeze-dried and cooked without soaking.
The determinations of phytate and tannin were carried out on the raw bean, the soaking water and on the differently processed beans, the results being presented in Table 3. With respect to the phytate content, it can be seen that treatments with soaking resulted in a reduction of these compounds. A reduction of 20.9% was obtained for BNS, 60.8% for BSNW and 53.0% for BSW, as compared to the raw bean. A similar result was obtained by other authors (3) when comparing the data of the raw bean (15.79 mg/g) and soaked and cooked bean (8.34 mg/g), showing a reduction of 47.18% of the phytate content. According to these authors, this reduction could be explained by the heat treatment (cooking), where an intensification of the fermentative effect occurs with respect to the phytic acid, decreasing the total content.

Phytic acid is an abundant constituent of plants, accounting for from 1 to 5% of the weight of legumes (21). The data for phytate found in Table 3 are in agreement with those of Cheryan (21), who observed that the phytate content of raw beans was 1.38% and also those of Barampama and Simard (3), who found 16.50 mg/g phytate in bean. The cooking methods play an important role in the reduction of phytate. Other authors (2, 22) have shown that soaking, fermentation, autoclaving and boiling, all reduce the phytate content, by the action of the heat treatment or by the activation of phytases which act on phytic acid substrates, decomposing them.

With respect to the tannin contents, it was shown that the cooking process effected a pronounced reduction of these components. This fact was also observed by other authors (3, 23). The following reductions were obtained: BNS 86.6%, BSNW 88.7% and BSW 89.0%. This reduction is probably due to diffusion of this anti-nutrient into the water or by the formation of insoluble complexes between proteins and tannins. Salunkhe et al. (24) found a decrease in the tannin content in soaked beans. Considering these facts, it was concluded that the soaking procedure prior to cooking, with or without the use of the non-absorbed soaking water, did not present a significant effect on the reduction of tannin, since the thermal process alone is capable of causing a considerable reduction in the content of this anti-nutritional factor.

### Biological assay

The results obtained for NPR and digestibility of the animals submitted to the experimental diets, are presented in Tables 4, 5 and 6. For the aproteic diet there was an average loss of weight of 4.7 ± 1.3 g and a diet consumption of 38.8 ± 3.6 g. The body weight gains and NPR values of rats fed casein diets were larger than those fed diets containing cooked beans as a source of protein (25), being the major cause for this fact probably the smaller amounts of the sulfurated amino acids contained in the beans, in relation to casein (Table 2). It was also observed that with the casein based diets, there were no significant differences between the values for the NPR, indicating that neither the phytate nor the tannin present in the non-absorbed soaking water, directly affected the net utilization of casein which is a protein of high quality. With respect to the bean treatments, it was verified that they did not differ between themselves with respect to weight gain, but NPR values in BSNW was significantly lower than that in BNS, however both values are suitable for these legumes.
Table 5. Determination of ingested nitrogen (IN), faecal nitrogen (FN), and absorbed nitrogen (AN) in recently weaned rats fed diets containing casein or the common bean as protein sources.

| Treatments   | IN (mg/5 d) | FN (mg/5 d) | AN (mg/5 d) |
|--------------|-------------|-------------|-------------|
| Casein control | 944.5 ± 179.8<sup>a</sup> | 51.6 ± 13.1<sup>a</sup> | 893.0 ± 169.8<sup>a</sup> |
| CSS<sup>*</sup> | 973.1 ± 70.5<sup>a</sup> | 58.2 ± 19.6<sup>a</sup> | 915.3 ± 58.6<sup>a</sup> |
| BNS<sup>**</sup> | 702.8 ± 147.9<sup>b</sup> | 261.6 ± 57.4<sup>b</sup> | 442.0 ± 102.5<sup>b</sup> |
| BSNW<sup>***</sup> | 646.8 ± 105.5<sup>b</sup> | 271.7 ± 54.7<sup>b</sup> | 373.9 ± 56.0<sup>b</sup> |
| BSW<sup>****</sup> | 611.9 ± 93.6<sup>b</sup> | 259.5 ± 51.2<sup>b</sup> | 351.0 ± 49.2<sup>b</sup> |

<sup>a,b</sup> Different letters in the same column indicate a statistical difference (p<0.05).
<sup>*</sup> Casein + soluble solids.
<sup>**</sup> Bean cooked without soaking.
<sup>***</sup> Bean cooked without non-absorbed soaking water.
<sup>****</sup> Bean cooked with non-absorbed soaking water.

Table 6. Protein digestibility in recently weaned rats fed on diets containing casein and the common bean as protein sources.

| Treatment     | Apparent digestibility (%) | Digestibility corrected by non-protein diet (%) |
|---------------|----------------------------|-----------------------------------------------|
| Casein control| 93.4 ± 0.8<sup>a</sup>     | 94.6 ± 0.8<sup>a</sup>                         |
| CSS<sup>*</sup> | 93.0 ± 1.6<sup>a</sup>     | 94.1 ± 1.8<sup>a</sup>                         |
| BNS<sup>**</sup> | 59.8 ± 1.3<sup>b</sup>     | 61.4 ± 1.4<sup>b</sup>                         |
| BSNW<sup>***</sup> | 57.0 ± 1.7<sup>bc</sup>   | 58.7 ± 1.8<sup>bc</sup>                        |
| BSW<sup>****</sup> | 55.8 ± 3.4<sup>c</sup>    | 57.5 ± 3.5<sup>c</sup>                         |

<sup>a,b,c</sup> Different letters in the same column indicate a statistical difference (p<0.05).
<sup>*</sup> Casein + soluble solids.
<sup>**</sup> Bean cooked without soaking.
<sup>***</sup> Bean cooked without non-absorbed soaking water.
<sup>****</sup> Bean cooked with non-absorbed soaking water.

Table 5 shows the data for faecal nitrogen (FN), ingested nitrogen (IN) and absorbed nitrogen (AN) in the biological assay carried out with the common bean. The animals fed on diets with the common bean as protein source excreted much more faecal nitrogen (4–5 times) than those fed on casein based diets. The differences found in the values for faecal nitrogen could be due to the lower digestibility associated with a higher faecal excretion of endogenous nitrogen in animals consuming bean based diets, even after cooking, as already observed by other researchers (25, 26), also taking into account the fact that tannins interfere in the digestion of nutrients by stomachal competition, which explains in part this increase in the excretion of endogenous nitrogen by these animals (27).

According to Marquez and Lajolo (26), there are various reasons, still not clearly explained, for the increase in faecal nitrogen excretion. The occurrence of probable interactions with the bean proteins or of the digestive enzymes with non-protein components present in the bean, such as fibre, carbohydrate and tannins (28), decrease the digestibility of the protein of these legumes. However, the tannins can be reduced by from 37.5 to 77.0% by procedures such as soaking and cooking (29).

The common bean proteins presented a lower digestibility than casein (Table 6), as widely described in the literature (30). However, it was shown that the domestic procedures vary between themselves, conferring greater digestibility on the non-soaked bean, as compared to the digestibility of the soaked beans, especially that in which the bean was cooked with the non-absorbed soaking water, this being in agreement with the literature (31).

The values obtained for the apparent digestibility and digestibility corrected by non-protein diet of casein were respectively 93.0–94.6%, whilst for the common bean these values were 55.8–61.4%, in accordance with the literature (25). The lower values found for the bean, amongst other factors, confirm the possible role of greater endogenous nitrogen excretion, in the quality of the protein of legumes (25, 32). Mendez et al. (33) suggest that the food fibre present in legumes could form indigestible complexes with proteins and amino acids, mainly during heat treatment, reducing the availability of the protein nitrogen for absorption and, consequently, decreasing the digestibility of the protein of legumes.

The lower digestibility in diets containing the common bean, can not be exclusively explained by the presence of anti-nutritional factors. The mechanisms are still not clear, but protein-tannin interactions (34) apparently affect the digestibility and availability of amino acids. In addition, other factors, such as the fibres, which cause an increased excretion of endogenous nitrogen, could have been left unconsidered.

Considering the results obtained for the phytate and tannin contents in the variously treated common bean samples, as presented in Table 3, there is apparently no explanation for the fact that the bean cooked without soaking showed a greater protein digestibility than that of the soaked beans (Table 6). One could consider the possibility that this be explained by the resulting structure of the soaked beans, and not by the discarding or otherwise of the non-absorbed soaking water, since this did not result in a difference in digestibility. This fact was observed by Melito and Tovar (35) in a study on soaked beans, suggesting a physical inaccessibility to
the digestive enzymes by the formation of biopolymers of starch and protein, surrounding the cell walls in protein fractions.

The greater question of possible health risks caused by anti-nutritional factors is surrounded by doubts, due to a lack of knowledge of the levels of tolerance, the degree of variation in individual risk and the influence of environmental factors on the desintoxication capacity of the organism, since slight chronic damage caused by the prolonged ingestion of anti-nutritional factors is very difficult to evaluate and the effects of these factors on human health are still highly questionable due to a lack of studies in the area. Many controversies exist in studies on "in vivo" bioavailability, due to the extrapolation of the results of experimental systems to human beings consuming complex diets.

From an analysis of the results obtained in the biological assay with casein and the common bean, one can conclude that soaking reduced the phytate content by up to 61%, whether the non-absorbed soaking water was used or not. The heat treatment alone, without soaking, was capable of reducing the phytate level by 21%. With respect to the tannins, soaking showed no effect, since the heat treatment alone reduced their content by up to 87%. Thus soaking and the anti-nutritional factors removed did not interfere in the growth indexes, neither for the casein nor for the common bean, mainly with respect to weight gain. NPR and apparent digestibility and digestibility corrected by non-protein diet.

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