Effect of Heat Treatment on the Toxicity and Nutritive Value of Dry Bean (*Phaseolus vulgaris* var. Rosinha G2) Proteins

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Proximate composition, amino acid pattern, and toxicity of an unheated dry bean flour and of six other materials obtained by fractionation of the flour were studied. When fed to weanling rats the flour and all six fractions were toxic. Most of the toxicity was eliminated by heating the soaked beans 2.5 min at 97 °C, but maximum PER was attained after 10 min at the same temperature. Not all trypsin inhibitor and phytohemagglutinin activities had been eliminated by any of these treatments. Autoclaving (121 °C, 15 min) decreased the availability of lysine in the whole flour and in the water-insoluble solids by 36.7 and 29.3%, respectively, whereas it did not affect significantly that of the other fractions. Heating at 121 °C for 7.5 min was deleterious to the nutritive value of the isolated protein fractions.

Leguminous seeds constitute an important source of food protein and energy for a large sector of the world population. The increasing interest in the food legumes has been demonstrated by several international symposia on this topic in the last decade (Milner, 1973; Wall, 1973; Jaffé, 1977).

In Brazil and in other South American countries the common bean (*Phaseolus vulgaris*) is by far the most consumed leguminous seed in human diets.

The main problems related to the biological utilization of bean proteins are as follows: (a) deficiency of sulfur-containing amino acids, (b) presence in the seeds of several antinutritional and toxic components, and (c) low digestibility of the bean proteins.

In the present paper, the relative toxicities of the raw bean flour and of several fractions obtained from the flour are reported. Data are also presented on the heat inactivation of the toxic components and on improvement of the proteins' nutritive values by heat treatment.

MATERIALS AND METHODS

The dry bean (var. Rosinha G2) used in this study was obtained from the leguminous plant section of the Agronomic Institute at Campinas, State of São Paulo, Brazil.

The bean flour was prepared by grinding the seeds in a hammer mill until all the material passed through a 70-mesh screen.

Fractionation of the flour was accomplished by extraction either with distilled water or with a 2% NaCl solution. The fractions F1, F2, and F3 were obtained from the NaCl extracts by dialysis and freeze-drying (F1) or separating, by centrifugation (10000*g*, 20 min), the supernatant containing the albumin (F2) from the water-insoluble globulin fraction (F3). The water-extractable solids (F4) were prepared by freeze-drying the undialyzed water extracts. The dialyzable solids (F5) were obtained by dialysis of the water extracts and freeze-drying of the dialysate. The residue of the water extracts gave the water-insoluble solids (F6).

Crude protein, ash, and crude fiber contents were determined on all samples according to the procedures described by the AOAC (1975). Nonprotein nitrogen was determined by the method of Becker et al. (1940). Total lipid was determined gravimetrically by evaporation of the solvent from a petroleum ether extract. Carbohydrate was calculated by difference. Amino acid determination was performed by the method of Spackman et al. (1958), using a Beckman 120C amino acid analyzer, and the procedure recommended by the instrument manufacturer. Tryptophan was determined in the enzymatic hydrolysate by procedure W of Spies (1968) and methionine by the method of Lunder (1973).

For the toxicity tests, 10 weanling male rats of the Wistar strain were used in each group. The rats were fed ad libitum with diets containing 10% of crude unheated protein and inspected daily. Food intake and body weight changes were recorded twice a week. The remainder of the composition of the diet was similar to that of the AOAC procedure (1975), taking into consideration the fiber and carbohydrate contained in the bean fractions.

Trypsin and chymotrypsin inhibitor activities were determined by the procedures of Kakade et al. (1969) and Kakade et al. (1970), respectively, using casein as substrate for both enzymes.

The procedure for testing phytohemagglutinin (lectin) activity was essentially that of Liener (1955) except for the final colorimetric reading which was replaced by serial dilution of the extract and visual detection of the hemagglutination. Activity was expressed as the reciprocal of the minimum concentration, in micrograms of protein per milliliter, capable of causing the reaction.

For the study of trypsin inhibitor and phytohemagglutinin inactivation, the following procedure was used. Samples of bean seeds were first soaked overnight in distilled water and then heat treated in boiling distilled water (97 °C) for various periods of time. Subsequently, the samples were frozen, freeze-dried, and ground to pass a 70-mesh screen. The water-soluble proteins were extracted from the flours and the trypsin inhibitor and phytohemagglutinin activities were determined in the water extracts.

Polyacrylamide disc gel electrophoresis was performed on the water extracts, using the procedure of Davis (1964).

Available lysine was determined by the colorimetric method of Kakade and Liener (1969).

Protein efficiency ratio (PER) was determined on all samples after various heat treatments according to the procedure described by the AOAC (1975).

RESULTS AND DISCUSSION

The proximate compositions of the bean flour and of six fractions (F1–F6) obtained from the flour are shown in Table I. Crude protein contents, on a dry basis, varied from 15 (F6) to 90% (F3). The lowest protein content was

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Table I. Proximate Composition of a Dry Bean (*Phaseolus vulgaris*) Flour and of Six Other Materials Resulting from Fractionation of the Flour (Dry Basis)

materials	crude protein (% N × 6.25), %	nonprotein nitrogen (% N × 6.25), %	ash, %	petroleum ether extractable material, %	crude fiber, %	carbohydrate,[b] %
flour[a]	26.3	3.7	3.2	2.0	5.1	63.4
F1	82.4	3.4	1.5	0.1	0.0	16.1
F2	70.3	7.8	3.4	0.0	0.0	26.3
F3	89.7	1.0	1.0	0.2	0.0	9.2
F4	50.2	10.9	9.0	0.8	0.0	40.1
F5	20.4	18.5	17.2	0.3	0.0	62.2
F6	15.1	0.6	0.9	1.4	7.7	74.9

[a] Bean ground to pass a 70-mesh screen; F1, total protein isolate; F2, albumin; F3, globulin; F4, water-extractable solids; F5, dialyzable solids; F6, water-insoluble solids. [b] By difference.

Table II. Amino Acid Composition of a Dry Bean Flour (*Phaseolus vulgaris*) and of Six Other Materials (F1-F6) Obtained by Fractionation of the Flour[a]

amino acid	amino acid composition, g/16 g of N						
	flour	F1	F2	F3	F4	F5	F6
Lys	7.7	7.9	7.5	8.0	7.7	1.5	8.7
Thr	5.2	5.5	6.5	4.8	4.8	1.0	5.2
Val	4.3	5.7	6.2	6.2	4.7	0.9	6.4
Met[b]	1.3	1.0	1.7	1.2	1.4	0.4	1.2
Ile	4.2	4.9	5.1	5.8	4.1	0.6	5.8
Leu	9.7	9.8	10.0	12.5	8.2	1.7	12.3
Phe	5.8	6.5	6.5	7.7	4.8	0.8	6.4
Trp[c]	1.3	1.7	3.2	1.2	1.4	0.3	0.6
His	2.6	2.8	2.5	3.3	2.8	1.5	4.2
Arg	5.5	4.5	4.3	5.6	6.7	8.2	6.9
Asp	17.1	16.8	16.6	20.3	16.8	10.9	18.5
Ser	7.6	7.6	7.7	8.6	6.0	0.7	7.9
Glu	24.8	17.8	16.6	24.2	19.2	23.6	23.8
Pro	3.9	3.8	4.2	4.0	3.4	0.0	4.7
Gly	4.6	4.1	5.0	4.7	3.7	1.0	5.8
Ala	4.6	4.6	5.2	4.8	2.4	1.5	5.3
$^1/_2$-Cys	1.4	1.3	1.6	1.1	1.3	0.8	1.7
Tyr	2.9	3.0	3.3	3.4	2.5	0.4	3.1
NH_3	2.0	2.1	2.1	3.9	2.2	2.4	1.9

[a] F1, total protein isolate; F2, albumin; F3, globulin; F4, water-extractable solids; F5, dialyzable solids; F6, water-insoluble solids. [b] Method of Lunder (1973). [c] Method of Spies (1967).

Table III. Toxicity of a Dry Bean (*Phaseolus vulgaris*) Flour and of Different Fractions of the Flour

materials tested	body wt change, g rat^{-1} day^{-1}	consumption of diet, g rat^{-1} day^{-1}	lethal period, days
flour	−1.6	2.5	4-8
flour, 1.5% Met[a]	−1.4	2.8	4-8
flour, 3.0% Met[a]	−1.1	2.9	4-9
dehulled bean flour	−1.8	3.0	3-7
flour extracted 8 times with 70% ethanol	−1.2	3.7	3-10
water-extractable solids (F4)	−2.4	3.8	5-9
water-insoluble solids (F6)	−0.9	2.0	10-14
albumin (F2)	−2.0	2.9	6-10
globulin (F3)	−1.1	4.4	12-23
total protein isolate (F1)	−1.5	3.0	5-9
dialyzable solids (F5)	−2.7	3.2	3-7

[a] Percent DL-methionine on protein basis.

found in the water-insoluble solids (F6) and the highest in the globulin fraction (F3). Nonprotein nitrogenous compounds ranged from less than 1% in the globulin fraction (F3) to 18.5% in the dialyzable solids (F5). Ash contents varied from 0.9 to 17.2%. The dialyzable solids (F5) had the highest ash content, followed by the water-extractable solids with 9%. Petroleum ether extractable material was low in all fractions since the integral flour had only 2% total lipids. Crude fiber was 5.1% in the bean flour and 7.7% in the water-insoluble residue; all the other fractions were essentially fiber-free.

The amino acid contents of the bean flour and of the different fractions (F1–F6) are given in Table II. It is known that the limiting essential amino acid in all bean varieties is methionine. The low content of cysteine is also of nutritional significance since methionine is utilized in cysteine biosynthesis when this amino acid is below the requirement for protein biosynthesis. Improving bean protein quality to its maximum value by adding methionine also requires added cysteine to spare the essential methionine. The amino acid profile of the bean flour is similar to reported values for other bean varieties (Moraes and Angelucci, 1971; Tandon et al., 1957; Kakade and Evans, 1965).

The essential amino acid compositions of the albumin and globulin fractions reported here differ substantially from those reported by Pant and Tulsiani (1969) for three varieties of bean (*Phaseolus*) cultivated in India. The values given by Pant and Tulsiani are considerably lower than ours for all essential amino acids, particularly for methionine, tryptophan, and lysine.

The amino acid composition of the dialyzable solids (F5) represents the free amino acids of the bean plus the amino acids contained in small peptides. The predominant amino acids in this fraction are arginine, aspartic acid, and glutamic acid. It is possible that the major part of glutamic and aspartic acids came, by the acid hydrolysis, from glutamine and asparagine, which are relatively abundant in all plant extracts.

Table III summarizes the results of the toxicity tests of the raw bean flour and of different fractions of the flour. All material tested was quite toxic as shown by death of the rats from the third to the 23rd day of the experiment. All materials, except the water-insoluble solids and the globulins, killed all the rats by the 10th day on the diets. For the water-insoluble solids and for the globulins the lethal periods were from 10 to 14 and from 12 to 23 days, respectively. Judging by the relative rate of death, the most toxic materials were the dehulled bean flour and the dialyzable solids, followed by the integral flour. The least toxic material was the globulin fraction. Similar toxicity had been reported by Bressani et al. (1963) and Kakade and Evans (1965) for a black bean and the navy bean (*P. vulgaris*) varieties, respectively.

Pusztai et al. (1975) detected toxicity in several kidney bean fractions. They found the albumin more toxic than the globulin fraction and attributed the higher toxicity to

Table IV. Activity of Antinutritional Factors in a Dry Bean (*Phaseolus vulgaris*) Flour and in Five Fractions of the Flour

materials	trypsin inhibitor activity, TUI/mg of protein	chymotrypsin inhibitor activity, ChUI/mg of protein	phytohemagglutinin activity, $1/(\mu g$ of protein $mL^{-1})^a$
flour	163.0	41.8	2.0
water-extractable solids	239.5	65.8	2.6
water-insoluble solids	17.4	19.2	0.8
albumin	376.5	115.0	8.3
globulin	63.8	23.7	4.0
total protein isolate	174.0	78.2	5.3

[a] Protein concentration capable of causing hemagglutination of trypsin-treated bovine red blood cells. Numbers are reciprocals of the actual concentrations.

the presence of one or more phytohemagglutinins in this fraction. Pusztai and Palmer (1977) purified, by affinity chromatography, a phytohemagglutinin which when added to a casein diet reduced the growth rate of the rat significantly; however, the animals did not die as happened when the integral raw bean flour was fed in the present work.

Jaffé et al. (1972) distinguished phytohemagglutinins with different degrees of toxicity in leguminous seeds.

It is not certain at the present time which is the lethal factor in the raw bean as well as in the different protein fractions extracted from the seeds. There are several known antinutritional factors which cause repression of growth and poor nutrient utilization of animal diets containing raw leguminous seeds. However, none of these factors when isolated and introduced into the rat diet caused death. Possible explanations are as follows: (a) the lethality of the raw bean could be caused by the combined action of several toxic components possibly including unidentified compounds and/or (b) the existence of one or more unidentified low molecular weight toxic substances which could bind more or less nonspecifically to various cell materials. The presence of toxic compounds in the dialyzable solids indicates there are low molecular weight toxic substances.

The activities of trypsin and chymotrypsin inhibitors and phytohemagglutinin of the bean flour and of five other fractions are shown in Table IV. The highest activities for all three antinutritional factors were found in the albumin fraction. Considerable hemagglutinating activity was also found in the globulin fraction, thus resulting in high activity in the total protein isolate (albumin plus globulin).

The effect of heat treatment (97 °C) on inactivation of the trypsin inhibitor and of phytohemagglutinin is shown in Figure 1. The data show quite clearly that heating water-soaked beans for 5 and 10 min in boiling water completely inactivated the inhibitor and the hemagglutinin, respectively, as measured in the water extracts. On the other hand, electrophoresis of the water-extractable proteins (Figure 2) clearly shows the disappearance of the two fastest running protein bands which correspond to trypsin inhibitors. This suggests that insolubilization and inactivation of the inhibitors took place as the heat

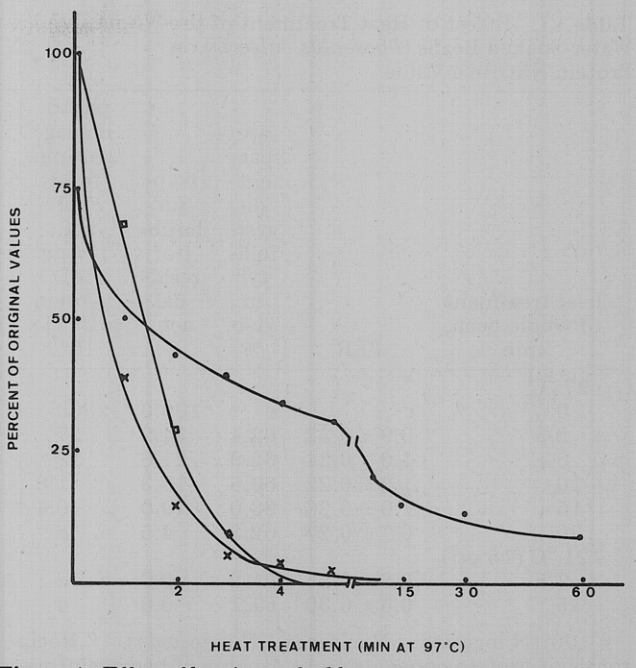

Figure 1. Effect of heating soaked beans on protein extractability and on trypsin inhibitor and phytohemagglutinin activities from a dry bean variety: (□–□) activity curve for trypsin inhibitors, (x–x) activity curve for hemagglutinin, (●–●) protein solubility curve.

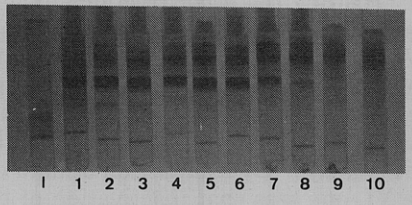

Figure 2. Electrophoretic pattern of water extracts from water-soaked beans (*Phaseolus vulgaris*) submitted to different heat treatments. (I) Trypsin inhibitor purified on a Sephadex G-100 column.

Table V. Effect of Heat Treatment on Available Lysine of a Dry Bean (*Phaseolus vulgaris*) Flour and of Six Fractions of the Flour

materials	available lysine after heat treatment,[a] min at 121 °C				
	0	5	15	30	60
flour	6.0	3.8	3.8	3.5	3.5
water-extractable solids	6.3	6.3	5.9	5.9	5.9
water-insoluble solids	4.1	3.2	2.9	2.9	2.9
albumin	6.3	6.1	6.1	5.7	5.7
globulin	6.5	7.1	6.5	6.2	6.2
total protein isolate	6.0	6.4	6.3	6.1	6.0
dialyzable solids	0.0	0.0	0.0	0.0	0.0

[a] Autoclaving under normal procedure (15 psi).

treatment increased. Alterations also occurred in the phytohemagglutinin band as the heat treatment increased.

The effect of autoclaving (121 °C) the bean flour and the fractions of the flour for various times on the availability of lysine is shown in Table V. By comparing the lysine data of Table V with that of Table II, one concludes that some 16–24% of the total lysine from undenatured whole bean flour proteins and from protein isolates was unavailable as determined by the trinitrobenzenesulfonate (TNBS) reaction. The lysine present in the dialyzable solids was very low and totally unavailable. Heating the

Table VI. Effect of Heat Treatment of the Whole Water-Soaked Beans (*Phaseolus vulgaris*) on Protein Nutritive Value

heat treatment of whole bean, min	PER	apparent digestibility[a] in vivo, %	trypsin inhibitor residual act., %	phytohemagglutinin residual act., [1/(μg of protein mL^{-1})] × 10^3[b]
97 °C				
0	[c]	[c]	100.0	2000
2.5	0.9 ± 0.32	62.4	34.2	27
5	1.0 ± 0.24	62.9	21.6	13
10	1.2 ± 0.22	63.5	13.3	1.6
15	1.0 ± 0.26	63.0	10.0	0.4
30	0.7 ± 0.27	62.3	4.5	[d]
121 °C (15 psi)				
7.5	0.8 ± 0.25	61.2	0.0	[d]
15	0.6 ± 0.30	61.1	0.0	[d]

[a] 100[(N ingested − N in feces)/(N ingested)]. [b] Reciprocals of protein concentrations capable of hemagglutination. [c] Death of the rats in 4–8 days. [d] Negative reaction for phytohemagglutinin.

whole flour and the water-insoluble solids at 121 °C for 15 min reduced lysine availability by 36.7 and 29.3%, respectively. Heating the other fractions under identical conditions did not affect lysine availability significantly.

Table VI shows the effect of heat treatment on nutritional values of the bean proteins (PER), on protein digestibility, and on inactivation of trypsin inhibitor and phytohemagglutinin in the water-soaked whole beans used for biological assays. By comparison of data of Figure 1 and Table VI, one sees that inactivation of the trypsin inhibitor and of phytohemagglutinin was accomplished after 30 min at 97 °C (Table VI) and in only 5 and 10 min, respectively, in Figure 1. A possible explanation for this is that in the heating experiments reported in Table VI a much larger quantity (3 kg) of beans was heated at once, decreasing the effectiveness of the heat treatment.

Optimum PER was found for the sample held 10 min in boiling water (97 °C). These data differ from those of Bressani et al. (1963), who reported 10–30 min at 121 °C as an ideal treatment for a black bean variety. It is interesting that in our experiments maximum protein efficiency ratio was found when considerable residual trypsin inhibitor and phytohemagglutinin activities were still present. It is also significant that 2.5 min of heating the soaked beans at 97 °C resulted in near maximum PER for the bean proteins. These data suggest either that the toxic substances in the bean (*P. vulgaris*) are destroyed by very light heat treatment or that under heat treatment they react very rapidly with some other cell material, thus becoming inactive. The data suggest also that the trypsin inhibitor and the phytohemagglutinin might not be the main toxic substances in the raw bean unless there are some very toxic trypsin inhibitors and phytohemagglutinins which are extremely heat labile, thus being destroyed very fast by the heat treatment.

Heating the soaked beans at 121 °C for 7.5 min was detrimental to PER, which is in agreement with Kakade and Evans (1965), who reported a decrease in PER by heating the bean longer than 5 min at 121 °C. Apparent digestibility was not significantly affected by heating the beans 30 min at 97 °C or 15 min at 121 °C (Table VI).

Table VII. Effect of Heat Treatment on the Nutritive Value of Protein Fractions Isolated from a Dry Bean (*Phaseolus vulgaris*) Flour Compared with Casein

protein fraction[a]	heat treatment at 121 °C, min	PER	digestibility in vivo, %
F1	7.5	0.7 ± 0.28	85.4
	15	0.6 ± 0.21	86.0
	30	0.5 ± 0.30	85.0
F2	7.5	0.7 ± 0.22	82.0
	15	0.5 ± 0.28	82.0
	30	0.45 ± 0.23	83.0
F3	7.5	0.6 ± 0.31	86.0
	15	0.4 ± 0.20	86.0
	30	0.2 ± 0.22	84.5
casein		3.4 ± 0.27	93.4

[a] F1, total protein isolate; F2, albumin; F3, globulin.

The effect of heat treatment (121 °C) on the nutritive values of the isolated protein fractions (F1, F2, F3) is shown in Table VII. The PER values of the proteins autoclaved at 121 °C for 7.5 min were considerably lower than those of the protein in the whole beans heated at 97 °C for a period of 5–15 min. The apparent digestibility was not affected significantly by autoclaving 7.5–30 min. The digestibility data of Tables VII and VI for the isolated proteins and for the crude protein (whole seeds) indicate that the digestibility of the isolated proteins is at least 20% higher than that of the crude protein. This might indicate that the low digestibility of the proteins in the flour is due to interactions and complexation of the protein with other substances, for example, the phenolic compounds.

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