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Use of Extrusion Technology To Overcome Undesirable Properties of Hard-To-Cook Dry Beans (*Phaseolus vulgaris* L.)

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The objective of this work was to study the effects of extrusion cooking on improving the functional and nutritive properties of dry beans that had been stored at 23 ± 3 °C and 65 ± 5% relative humidity for 12 months. After storage, the bean cooking time increased 4.5-fold, while texture and flavor deteriorated in quality. Water absorption, water solubility, and emulsifying capacity were the same for the extrusion cooked beans, before and after storage. Paste viscosity was slightly higher for the beans extruded shortly after harvest. Protein nutritive value of the freshly harvested beans was totally preserved in the stored beans submitted to extrusion cooking. Extruded mixed flours (1:3 w/w) of freshly harvested beans and rice or stored beans and rice, respectively, improved significantly in their water absorption capacity, viscosity of paste at 25, 90, and 50 °C, and nutritive value, but decreased in water solubility index and in emulsifying capacity, in relation to extruded flours of beans alone.

Keywords: *Dry beans; hard-to-cook beans; extrusion cooking; functional properties; nutritive properties*

INTRODUCTION

Dry bean, *Phaseolus vulgaris*, is the legume seed most consumed in Brazil. It is a good source of protein (18–27%) and energy (approximately 380 kcal/100 g of seeds) (Sgarbieri, 1989).

The quality of dry bean seeds may deteriorate very quickly as a function of time and condition of storage (Morris and Wood, 1956; Quast and Silva, 1977; Kon and Sanshuck, 1981; Antunes and Sgarbieri, 1979; Cunha et al., 1993). The factors responsible for bean deterioration have been identified as high water content in the beans or high relative humidity and temperature in the storage atmosphere. The main observed deteriorations have been hardness of the cotyledons and loss of cookability, deterioration of texture and flavor, and loss of nutritive value (Antunes and Sgarbieri, 1979; Mejia, 1982).

Some attempts have been made to prevent hardening (Molina et al., 1976; Rivera et al., 1989; Cunha et al., 1993) or to reverse hardening once it had occurred (Neme et al., 1975a,b; Reedy et al., 1979; Carvalho et al., 1991).

Effects of extrusion cooking on functional and nutritive properties of legume seeds have been reported (Cardoso Filho et al., 1993; Camire et al., 1990; Avin et al., 1992).

The objective of the present work was to use extrusion technology as an alternative way of overcoming such undesirable properties of hardened beans as unacceptable texture and to improve nutritive value by decreasing cooking time and by preparing mixed flours of beans and rice which should be of higher nutritive value then either bean or rice flour alone.

MATERIALS AND METHODS

Bean Samples. Brown beans of the cultivar Carioca 80 SH from two different harvests, 1 year apart, were used in this work. One sample (sample A) was used shortly after harvest, and the second sample (sample B) was stored in an air-conditioned room [23 ± 3 °C, 65 ± 5% relative humidity (RH)] in fiber bags for 12 months. Relative humidity was monitored by a recording hygrometer. Both samples originated from the same plot of land at the Agricultural Experimental Station of the Agronomic Institute of Campinas, at Campinas, State of São Paulo, and were grown under the same cultivation practices.

Cooking Time of Whole Beans. Cooking time was determined in a homemade puncturometer, according to the prototype and procedure described by Burr et al. (1968) and Burr (1973). Half cooking time was determined as the time (minutes) elapsed for half plus 1 of the 100 puncture units to penetrate the seeds, as suggested by Burr. Three replicates were done for each sample tested, and the cooking time was taken as the mean value of the replicates.

Hydration Capacity. Hydration capacity was studied by soaking 10 g of seeds in 100 mL of distilled water at room temperature. At 1 h intervals the seeds were drained and weighed until constant weight. Hydration capacity was expressed as hydrated weight divided by initial weight multiplied by 100.

Sensory Evaluation of the Bean Seeds and Extruded Flours. The seeds were prepared for the sensory tests by soaking in 4 times their weight of distilled water for 12 h and autoclaving (121 °C, 10 min) in the same soaking water. Samples, without any condiment, were presented to the tasters in beakers maintained at 40 °C, in booths illuminated with red light to mask possible color interference. A nonstructured 9 cm scale was used, anchored by the words noncharacteristic (left) and characteristic (right) for odor, taste, and texture. For bitter taste the words were none (left) and intense (right). Tests were done using eight tasters with six replications. The results were submitted to analysis of variance, and differences among means were analyzed by the Tukey test, according to the method of Gomes (1987).

Extrusion Cooking. For the extrusion, seed samples were first ground to pass a 0.76 mm screen using a TREU Series 63202 granulator mill. Before extrusion, water was sprayed on the flour under mechanical agitation to the specified moisture level. Moisture contents in the flours was conditioned to be 21.5% for the bean flours and 18.5% for the blends of 1:3 w/w bean and rice flours. These conditions were adopted after several preliminary tests to evaluate the machine

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performance as well as appearance and overall quality of the products. The extrusion was performed in a Brabender single-screw laboratory extruder, Model GNF 101412, electrically heated and provided with a vertical helix feeder of variable speed. The type of screw used was of constant pass and diameter with 38 cm of length and a 1.9 cm diameter.

Other conditions of extrusion were as follows: temperature of the first zone, 80 °C; temperature of the second and third zones, 190 °C; rotation of the screw, 100 rpm; die diameter, 3 mm: and compression ratio, 3:1.

Proximate Composition. Moisture content was determined by procedure 44-15A (AACC, 1976) and ash according to procedure 08-01 (AACC, 1976). Total lipid was determined following the method of Bligh and Dyer (1959). Total protein (N × 6.25) was determined according to the Kjeldahl semimicro method, and carbohydrate was determined by difference. Metabolizable energy (kilocalories per 100 g) was calculated using the energy values of 4 kcal/g for protein and carbohydrate and 9 kcal/g for lipids. Net dietary protein in calories percent (NDP cal %) was calculated using the following expression:

$$\text{NDP cal \%} = \frac{\text{protein calories}}{\text{total calories}} \times \text{NPU \%}$$

Water Absorption and Water Solubility Indices. The water absorption and the water solubility indices in the flours were determined according to the method described by Anderson et al. (1969).

Emulsifying Activity. Emulsifying activity was determined by an adaptation of the method of Dench et al. (1981). Five grams of extruded sample was suspended in 40 mL of 0.5 M NaCl solution and mixed well for 15 min, and the volume was adjusted to 50 mL. To this was added 50 mL of soy oil, and the combination was mixed in a Janke-Kunkel Ultra Turrax homogenizer under maximum velocity for 3 min. The emulsion was divided into two equal portions in centrifuge tubes and centrifuged at 1300*g* for 5 min. The calculation was done by dividing the height of the emulsified layer by the total height and multiplying by 100.

Viscosity Properties. A Brabender viscoamylograph and the technique described in procedure 21-10 (AACC, 1983) were used. Determinations were done at a concentration of 15%, and the moisture contents of the flours were corrected to 14%. The temperature was increased at 1.5 °C/min from 25 to 90 °C, held for 20 min, and cooled at 1.5 °C/min to the final temperature of 50 °C.

Trypsin Inhibitor Activity. The method of Kakade et al. (1969) was employed using *n*-benzoyl-DL-arginine-4-nitroanilide hydrochloride (BAPNA) at a concentration of 0.3 g/L in Tris buffer as substrate.

Hemagglutinating Activity. The procedure described by Junqueira and Sgarbieri (1981) was used.

Amino Acid Determination. Acid hydrolysates (6 N HCl, 105 °C, 22 h) were analyzed by ion exchange chromatography in a Beckman 119 CL amino acid analyzer.

Biological Assays. Protein nutritive properties were evaluated by PER and nitrogen balance procedures. Four experimental diets were prepared in powdered form and designated A–D, containing the following products as sources of dietary protein. Diet A contained the flour of beans extruded shortly after harvest. Diet B contained the flour of beans extruded after 12 months of storage at 23 ± 3 °C and 65 ± 5% RH. Diet C contained the extruded mixed flour (1:3 w/w) of bean flour (sample A) and rice flour. Diet D contained the extruded mixed flour (1:3 w/w) of bean flour (sample B) and rice flour. The diets were prepared to contain 10% protein, 8% vegetable oil, 4% mineral mix (AOAC, 1975), and 2% vitamin mix (NBC, 1977/1978). The contents of oil and carbohydrate of the beans and the rice were taken into consideration in the calculation of the diets, whereas the contents of fiber, vitamins, and minerals of the beans and rice were disregarded. A mixture of starch and sucrose (3:1 w/w) made up the remaining 76% of the diets.

Groups of five Wistar male rats were used for the various trials, with the following respective initial average body weights: A, 51.4 ± 5.4 g; B, 52.7 ± 6.8 g; C, 51.7 ± 4.9 g; D, 53.1 ± 6.5 g; casein control diet, 53.8 ± 9.2 g.

Table 1. Some Physical and Sensory Properties of Dry Bean (*P. vulgaris*) Seeds, Cultivar Carioca 80 SH, with Different Storage Times[a]

property	sample A	sample B
half cooking time (min)	34.5 ± 6.4^b	158.0 ± 4.2^a
texture	6.0 ± 0.3^a	4.1 ± 0.3^b
taste	8.4 ± 0.2^a	7.5 ± 0.3^b
odor	8.2 ± 0.2^a	8.0 ± 0.3^a
color	light brown	dark brown
hydration capacity (g of H_2O/100 g of seed)	109.6	103.4

[a] Different superscript letters within rows indicate statistically significant differences ($p \leq 0.05$). Sample A, analyzed shortly after harvest; sample B, stored at laboratory environmental conditions (23 ± 3 °C; 65 ± 5% RH) for 12 months.

Table 2. Sensory Properties of Extruded Flours of Dry Beans (*P. vulgaris*), Cultivar Carioca 80 SH, with Different Storage Times[a]

property	sample A	sample B
texture	7.08 ± 0.49^a	6.92 ± 0.47^a
taste (characteristic)	6.76 ± 0.45^a	5.00 ± 0.74^b
odor	6.98 ± 0.33^a	6.35 ± 0.46^a

[a] Different superscript letters within rows indicate statistically significant differences ($p \leq 0.05$). Sample A, analyzed shortly after harvest; sample B, stored at laboratory environmental conditions (23 ± 3 °C, 65 ± 5% RH) for 12 months.

The rats were fed *ad libitum* for 28 days and maintained in screened-bottom stainless steel cages to prevent them from eating their feces. Water was also offered *ad libitum*. Environmental temperature was maintained at 21 ± 2 °C with alternating periods of 12 h of light and 12 h of darkness. Food consumption was recorded every other day and body weight at the end of every week. During the second week of the diet, the animals were maintained in metabolic cages to permit collection of feces and urine for a period of 5 days for the calculation of nitrogen balance.

RESULTS AND DISCUSSION

In Table 1, cooking time, hydration capacity, texture, and flavor characteristics of a recently harvested whole bean sample are compared with those of a bean that had been stored for 12 months at 23 ± 3 °C and 65 ± 5% RH. Half cooking time increased from 34.5 to 158 min, a 4.5-fold increase for the stored sample. Hydration capacity of the whole beans was not altered significantly.

Texture of the stored beans, as measured by a panel of trained tasters, deteriorated significantly. Taste score also decreased significantly. A bitter taste was perceived more strongly in the stored sample compared with the beans cooked shortly after harvest. There was also a darkening of color, mainly in the seed coat. Odor did not change significantly.

Table 2 compares texture, taste, and odor attributes of extruded flours of beans shortly after harvest and after 1 year of storage, under the specified conditions. No significant differences were found for texture and odor. However, the score for characteristic bean flavor was significantly inferior for sample B, beans that had been stored for 12 months. As for the whole beans, the sensory panel perceived a bitter-tasting component in the taste of the extruded flour of sample B. We have no explanation for the appearance of bitter taste in the stored beans. One might speculate that it could be the

Table 3. Intensity of Bitter Taste of Extruded Flours of Dry Beans (*P. vulgaris*), Cultivar Carioca 80 SH, with Different Storage Times and Extruded Blended Flours of the Bean Flours and Rice Flour[a]

sample[b]	intensity of bitter taste[c]	sample[b]	intensity of bitter taste[c]
A	4.39 ± 0.22^{a}	C	3.00 ± 0.97^{b}
B	4.70 ± 0.30^{a}	D	2.87 ± 0.31^{b}

[a] Different superscript letters with the column indicate statistically significant differences ($p \le 0.05$). [b] A, flour of dry beans extruded and analyzed shortly after harvest; B, flour of dry beans extruded and analyzed after 12 months of storage (23 ± 3 °C, 6 ± 55% RH); C, extruded blended flour of sample A and rice flour (1:3 w/w); D, extruded blended flour of sample B and rice flour (1:3 w/w). [c] Bitter intensity: 0 = none; 9 = intense.

Table 4. Proximate Percent Composition and Protein/Energy Balance for Different Extruded Samples

determination	sample[a] A	B	C	D
moisture (%)	3.61	3.82	4.82	5.89
protein (% N × 6.25)	22.56	20.62	12.56	12.31
total lipid (%)	2.20	2.10	1.16	1.12
ash (%)	3.90	3.73	1.28	1.28
carbohydrate (difference)	67.73	69.73	80.31	79.40
energy (kcal/100 g)	380.96	380.30	381.92	376.92
NDP cal %	15.56	15.04	9.68	9.08

[a] A, bean sample analyzed shortly after harvest; B, bean sample analyzed after 12 months of storage (23 ± 3 °C; 65 ± 5% RH); C, blend (1:3 w/w) of sample A with rice; D, blend (1:3 w/w) of sample B with rice.

result of proteolysis and release of bitter peptides or even lipoxidation with consequent formation of bitter degradation products. Intensity of the bitter taste of the extruded flours A–D was evaluated in a separate test, and the results are shown in Table 3. When samples A and B were compared only for the bitter taste, no statistical difference was detected. However, when either sample A or B was compared with the blended flours C and D, it was found that bitter taste was significantly milder in flours C and D, which were composed of 75% rice.

Proximate composition, caloric value, and net dietary protein in calories (percent) are shown in Table 4 for the beans extrusion-cooked shortly after harvest (sample A), for the beans extrusion-cooked after 1 year of storage (23 ± 3 °C; 65 ± 5% RH), for extrusion-cooked blend (1:3 w/w) of sample A and rice, and for extrusion-cooked blend (1:3 w/w) of sample B and rice. The blended flours (samples C and D) retained more water than the extruded bean samples A and B. This was a reflection of the higher starch content introduced by the rice flour in the mixture. Protein content in the blended flours was roughly only 55% that of the bean flours alone, due to the high proportion of rice in the mixture and low concentration of protein in the rice. Total lipid content was approximately halved with the introduction of rice and the mineral content decreased to less than half that of the beans in the blended flours. Carbohydrate was significantly higher in the blended flours C and D as compared to the bean flours A and B.

Caloric value (kilocalories per 100 g) was roughly the same in the bean flours and in the blended flours. Nevertheless, the utilizable protein to calories ratio (NDP cal %) was significantly lower and nutritionally more adequate in the bean–rice blended flours. According to the FAO/WHO Expert Group (1965), the NDP cal % should be in the range of 8–12 for infants and 6–8 for adults.

Table 5. Amino Acid Profile of Extruded Dry Bean (A, B) and Extruded Mixtures (1:3 w/w) of Dry Bean and Rice

amino acid (g/16 g of N)	samples A	B	C	D
aspartic acid	15.1	13.2	9.4	8.6
threonine	4.9	4.0	4.0	3.9
serine	6.8	5.9	5.0	5.2
glutamic acid	23.5	18.3	21.1	19.1
proline	4.1	3.9	4.1	3.7
glycine	4.3	4.0	4.1	3.7
alanine	4.5	4.0	4.8	4.4
half-cystine	0.6	0.5	0.8	0.7
valine	5.3	4.5	4.7	4.7
methionine	1.2	1.1	1.8	1.8
isoleucine	4.5	3.8	4.2	3.9
leucine	9.2	7.2	7.4	7.2
tyrosine	3.2	2.8	3.4	3.4
phenylalanine	6.1	4.8	4.6	4.4
lysine	6.1	5.7	4.3	4.0
histidine	4.4	2.4	2.1	2.1
arginine	6.5	5.2	6.6	6.3

[a] A, bean, recently harvested; B, bean stored for 12 months (23 ± 3 °C; 65 ± 5% RH); C, blend (1:3 w/w) of sample A with rice; D, blend (1:3 w/w) of sample B with rice.

Table 5 presents the amino acid compositions of bean samples A and B and blended flours (1:3 w/w) of bean and rice. The addition of rice to the bean flour increased the content of sulfur amino acids and decreased aspartic acid and lysine contents. As a whole, the blended flours presented a better amino acid balance for growth, which is reflected in the higher protein nutritive value of the blended flours as compared to the bean flours (Tables 7 and 8). It is fortunate that in Brazil beans and rice are usually combined in the same meal, thus improving the protein value of the diet. It is apparent that the recently harvested beans (sample A) showed, in general, higher recovery of amino acids than the beans stored for 12 months (sample B). This suggests degradation of amino acids during storage since the heat treatment for both samples was the same; however, we have no clear explanation for these differences.

Some functional properties of the extrusiuon-cooked bean flours (samples A and B) and for the blended flours (samples C and D) are presented in Table 6. Water absorption capacity (grams of water per gram of sample) was much higher in the bean–rice flours than in the bean flour. The increase in water absorption with introduction of rice was likely due to the higher proportion of gelatinized starch in the blended flours (Table 4).

Similar results were described by Pilosof et al. (1982) and Han and Khan (1990). Pilosof et al. (1982) observed an increase in water absorption capacity with heat treatment which paralleled a decrease in nitrogen solubility index (NSI) in bean flours. They also observed that the heat effect on increasing water absorption was much greater in the bean flour as compared to a bean protein isolate. Similarly, Han and Khan (1990) reported that heat treatment of starch-rich fractions from beans and peas decreased their NSI and increased their water absorption capacities and cold paste viscosities. The starch-rich fractions were higher than a protein-rich fraction in water absorption capacity and cold paste viscosity, while the protein-rich fraction exhibited higher emulsifying and foaming capacity.

The higher water solubility indices and emulsifying activities (Table 6) of the bean flours (A and B) versus the bean–rice flours (C and D) are attributed to the higher protein concentrations in the former. Bean

Table 6. Some Functional Properties of Extruded Bean Flours and Extruded Mixed Flours of Bean and Rice

	sample^{a}			
property	A	B	C	D
water absorption index (g of H_2O/g of sample)	4.82 ± 0.09^{c}	4.78 ± 0.07^{c}	7.12 ± 0.05^{b}	7.80 ± 0.02^{a}
water solubility index (g of solids/g of sample)	0.30 ± 0.01^{a}	0.31 ± 0.00^{a}	0.09 ± 0.02^{b}	0.09 ± 0.01^{b}
emulsifying activity (%)	100.00 ± 0.00^{a}	100.00 ± 0.05^{a}	30.08 ± 2.71^{b}	40.76 ± 6.92^{b}
initial viscosity at 25 °C (AU)	240	240	520	580
minimum viscosity at 90 °C (AU)	60	40	110	90
final viscosity at 50 °C (AU)	110	40	200	100

[a] Different superscript letters within rows indicate statistically different results ($p \leq 0.05$) A, bean sample shortly after harvest; B, bean sample stored for 12 months (23 ± 3 °C; 65 ± 5% RH); C, blend (1:3 w/w) of sample A with rice; D, blend (1:3 w/w) of sample B with rice.

Table 7. Diet Intake, Protein Intake, Body Weight Gain, and PER of Rats Fed for 28 Days on Diets Containing the Extruded Flours A–D and Casein as the Only Source of Protein^{a}

protein source	diet intake (g)	protein intake (g)	body wt gain (g)	PER
A	316 ± 129.2^{a}	32.5 ± 3.0^{a}	49.7 ± 5.0^{b}	1.54 ± 0.14^{c}
B	303.1 ± 22.6^{a}	30.5 ± 2.3^{a}	46.1 ± 9.1^{b}	1.52 ± 0.33^{c}
C	404.9 ± 95.5^{a}	41.3 ± 9.7^{a}	96.7 ± 8.0^{a}	2.41 ± 0.36^{b}
D	351.5 ± 8.2^{a}	34.9 ± 0.8^{a}	92.9 ± 6.1^{a}	2.63 ± 0.13^{ab}
casein	373.3 ± 67.2^{a}	35.5 ± 6.4^{a}	110.4 ± 21.8^{a}	3.11 ± 0.09^{a}

[a] Different superscript letters within columns indicate statistically different result ($p \leq 0.05$). A, bean sample shortly after harvest; B, bean sample stored for 12 months (23 ± 3 °C; 65 ± 5% RH); C, blend (1:3 w/w) of sample A with rice; D, blend (1:3 w/w) of sample B with rice. Values are mean ± SD of five rats for a period of 28 days.

proteins, particularly the globulins and albumins, are good emulsifying and foaming agents (Deshpande et al., 1983).

Paste viscosities measured in a Brabender viscoamylograph are also shown in Table 6. Initial viscosity (25 °C), minimum viscosity (90 °C), and final viscosity (50 °C) were considerably higher in the bean–rice flours (C and D) than in the bean flours (A and B). This was expected in view of the higher starch contents in the composite flours. On the other hand, when flour from recently harvested beans (sample A) was compared with that of beans stored for 12 months (sample B) there was no difference in viscosity at 25 °C but viscosity was lower for the stored bean at 90 °C and 50 °C. The same general trend is observed for the blended flours C and D.

Low-viscosity broth was also observed when a hard-to-cook bean was cooked domestically for consumption (data not presented). This seems to be linked with the most acceptable hypothesis for explaining the hardening phenomenon wherein carbohydrates, particularly pectic substances from middle lamella, interact with divalent cations, strengthening cross-linkages between cotyledon cells and impeding their separation during cooking (Moscoso et al., 1984; Rozo et al., 1990).

Table 7 presents data on diet consumption, body weight gain, and PER for rats fed diets containing extruded bean flours A and B and extruded bean–rice flours C and D as the only sources of protein. A casein diet was used as control. There were no statistical differences in intake between diets, although intake tended to be higher for the casein and composite flour diets than for the bean flour (A and B) diets, mainly because the rate of growth was approximately double for the casein and the bean–rice flours compared to the bean flour diets A and B. Weight gains of rats receiving the composite flour diets approached that for the casein diet. It is important to notice that there was no difference between diet A (containing beans extruded shortly after harvest) and diet B (beans extruded after 12 months of storage) with respect to diet consumption, body weight gains, and PER. On the other hand, the PER values for the composite flour diets containing stored beans did not differ from that for casein ($p \leq 0.05$) and were superior to that for the composite diet containing beans extruded shortly after harvest (Table 7).

Extrusion cooking is considered to be a high-temperature short-time (HTST) heat treatment. Extrusion cooking lasts only 1–2 min, whereas conventional cooking normally requires 30–35 min for recently harvested beans and much longer (Table 1) for beans stored for several months under conditions conducive to hardening.

Antunes and Sgarbieri (1979), working with a different cultivar, reported important loss of protein nutritive value of beans stored for 6 months at three different conditions: 12 °C, 52% RH; 22–25 °C, 65–70% RH; and 37 °C, 76% RH. Cooking time in a domestic pressure cooker increased drastically with increase in storage temperature and relative humidity, which paralleled losses in bioavailable methionine and PER. PER and growth were recovered by complementation of stored beans with unavailable methionine. On the other hand, when the bean proteins were first extracted (protein isolate) and heated in an autoclave, loss of methionine bioavailability and PER decreased proportionally to the heat treatment (Antunes and Sgarbieri, 1980).

These reported data suggest that loss of protein nutritive value of hardened beans may be caused, in part, by extra heat treatment necessary to soften the beans. The high-temperature short-time treatment used in extrusion cooking seems to be able to accomplish cooking with less heat treatment, thus preserving the protein nutritive value.

Extrusion cooking was efficient in the inactivation of the bean lectins and trypsin–chymotrypsin inhibitors. Hemagglutinin titer decreased from 128 to 2, representing 98.5% inactivation. The trypsin–chymotrypsin inhibitor activity (units of trypsin inhibited per millogram of protein) decreased from 117 to 14, an inactivation of 88% of the activity. Complete inactivation of the trypsin inhibitors is very difficult to accomplish, and residual activity between 10 and 20% is normally found in processed foods (Anderson et al., 1979).

Results of the nitrogen balance study are shown in Table 8. The most evident differences are observed in

Table 8. Nitrogen Balance and Protein Utilization for Extruded Bean (A and B) Flours and Extruded Blended (1:3 w/w) Bean and Rice Flours (C and D)

	protein source[a]				
determination	A	B	C	D	casein
nitrogen intake (mg)	734 ± 91^{c}	780 ± 112^{c}	1193 ± 141^{ab}	1096 ± 68^{ab}	1023 ± 205^{a}
fecal nitrogen excretion (mg)	219 ± 24^{a}	216 ± 48^{a}	233 ± 31^{a}	225 ± 17^{a}	87 ± 16^{b}
urinary nitrogen	33 ± 17^{c}	26 ± 37^{c}	80 ± 22^{ab}	120 ± 36^{a}	67 ± 89^{b}
apparent protein digestibility (%)	70.1 ± 1.0^{c}	72.3 ± 4.7^{c}	80.5 ± 0.8^{b}	79.4 ± 1.5^{b}	91.5 ± 0.4^{a}
apparent biological value (%)	93.6 ± 2.7^{a}	95.3 ± 4.6^{a}	91.6 ± 2.4^{a}	86.2 ± 3.7^{a}	93.8 ± 6.0^{a}
net protein utilization (%)	66.2 ± 1.3^{b}	68.9 ± 5.9^{b}	73.8 ± 1.9^{b}	68.5 ± 3.4^{b}	85.8 ± 5.3^{a}

[a] Different superscript letters within rows indicate statistically significant differences ($p \leq 0.05$). A, bean sample extruded shortly after harvest; B, bean sample extruded after storage (23 ± 3 °C; 65 ± 5% RH) for 12 months; C, extruded blend (1:3 w/w) of sample A and rice; D, extruded blend (1:3 w/w) of sample B and rice. Results are means ± SD of five rats for 5 days.

the apparent protein digestibility. Digestibility of the extruded bean flour protein was significantly lower than that of extruded blended flour C and D proteins, which was also lower than casein digestibility. The low digestibility of dry bean seed protein is a well-established fact (Sgarbieri and Whitaker, 1982; Sgarbieri, 1989). On the other hand, the apparent biological value, which measures the degree of utilization of the absorbed amino acids, did not differ among all five dietary protein sources. Nevertheless, the NPU, which gives the overall dietary protein utilization, was superior for casein but did not differ statistically among the bean and the blended bean–rice flours. This is not readily explainable, since total body weight gain and PER for the blended flours were significantly greater than when the bean flours were the only sources of protein. For some reason, urinary excretion of nitrogen for the rats fed the blended flour diets was significantly higher than for those fed the bean diets. The comparison of data of Tables 7 and 8 suggests that growth rate and PER in these experiments were influenced by dietary factors other than nitrogen retention and utilization, which may be explained by differences in the rice and bean composition and physiological properties. Differences in the contents and nature of the dietary fiber and starch fraction may have been important factors.

In conclusion, the indices of protein nutritive value presented in Tables 7 and 8 suggest that extrusion cooking entirely preserved the nutritive value of beans stored for 12 months, which may, in part, be attributed to the shorter heating time employed in the extrusion cooking. On the other hand, extruded bean–rice flours (1:3 w/w) exhibited considerably improved protein nutritive value, texture, and flavor, including a significantly decreased bitter taste.

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