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Protein Nutritive Value of a New Cultivar of Bean (*Phaseolus vulgaris* L.)

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The objective of this paper was to report the proximate composition, amino acid profile, and protein nutritive value of a new cultivar of dry bean (Carioca 80), which has been developed by crossing the Brazilian cultivar Carioca with the variety Cornell 49-242. In addition to improved productivity and resistance to rust and anthracnosis, the new cultivar presented methionine bioavailability of 57%, digestibility around 70%, and biological value from 75 to 80%. The limiting amino acid was methionine. Supplementation with 0.3% of methionine, on a protein basis, raised the biological value to 92%, superior to that of casein utilized as reference. The PER at 10% dietary protein did not differ from that of cultivars Carioca and Aeté 3 used in the PER assays for comparison, but at 21% bean protein the PER of Carioca 80 was 40% higher.

Dry beans are a very important source of calories and proteins for people in many countries. In Brazil legume seeds, excluding soybean and peanuts (FAO, 1966), contribute daily intake of 220 kcal and 14.8 g of protein. *Phaseolus* proteins have been characterized as having low nutritive value due to limiting amounts of sulfur-containing amino acids, low digestibility, low bioavailability of essential amino acids, presence of toxic proteins, and other antinutritive factors (Sgarbieri and Whitaker, 1982; Sgarbieri and Garruti, 1986; Sgarbieri et al., 1979; Durigan et al., 1987a,b). It has also been shown that whole beans and isolated bean proteins, both raw and heat-treated, stimulate considerably greater excretion by the rat of endogenous nitrogen, when compared with a casein or protein-free diet (Oliveira and Sgarbieri, 1986a,b).

In this paper we report a new dry bean cultivar with higher protein digestibility, high methionine bioavailability, and higher biological value than the Brazilian cultivars already studied.

MATERIALS AND METHODS

Cultivar. A new cultivar was developed at the Agronomic Institute of Campinas, SP, Brazil, named Carioca 80 resulting from crossing the cultivar Carioca with the variety Cornell 49-242. Plant selection was initiated with the F_2 generation and continued with the F_3 after artificial inoculation of the fungus responsible for anthracnosis. In this generation no segregations were observed for the gene *Are*; therefore, the progenies in F_4 were planted under field conditions (Pompeu, 1979). Further selection was performed in the F_5 generation for characteristics like resistance to the rust fungus and to the common mosaic virus, thus initiating the evaluation for productivity. The cultivar Carioca 80 resulted, finally, from three isogenic strains identified by the numbers 10-5-1, 10-6-2, and 10-9-1 (Pompeu, 1982). The new cultivar was 10–15% more productive than the cultivar Carioca, besides being resistant to the rust fungus, to anthracnosis, and to common mosaic virus. It also presented improved nutritional properties.

Cooking Conditions. Beans were soaked in distilled water at room temperature (12–48 h) and then cooked under pressure (15 psi, 40 min) in a pressure cooker. Cooked beans, with or without soaking water, were frozen and freeze-dried prior to grinding for use in the experiments.

Chemical and Biochemical Determinations. Total protein (% N × 6.25), total lipids, crude fiber, and ash contents were determined by AOAC (1980) procedures. The neutral detergent residue (NDR) was determined by the method of Van Soest and Wine (1967). The pepsin pancreatin residue was obtained by the procedure of Hellendoorn et al. (1975) using pepsin (2000 U/mg of protein) and pancreatin (350 FIP-U/g of protease; 7500 FIP-U/g of lipase; 7500 FIP-U/g of amylase) both from Sigma Chemical Co. Amino acids were determined by ion-exchange chromatography (Spackman et al., 1958) on a Beckman Model 119 CL amino analyzer (Beckman Instruments, 1977). Methionine was also determined by the sodium nitroprusside colorimetric reaction of a pepsin/pancreatin digest according to Tannembaum et al. (1969) as modified by Badiale (1979) and by the reaction of BrCN of a bean flour suspension followed by gas–liquid chromatography of the reaction byproduct CH_3SCN, according to Apostolatos and Hoff (1981). Trypsin inhibitor activity was measured in the raw and cooked bean extracts by the method of Kakade et al. (1969) with casein as enzyme substrate. Lectin activity (hemagglutination) was performed in raw and cooked bean extracts by the serial dilution procedure using

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Table I. Proximate Percent Composition of Main Components

component	compn,[a] %
total crude protein (N × 6.25)	21.80 ± 0.60
total lipids	1.23 ± 0.05
ash	3.48 ± 0.06
crude fiber	5.20 ± 0.03
neutral detergent residue (NDR)	
raw sample	40.13 ± 6.18
cooked sample[b]	14.06 ± 4.92
pepsin/pancreatin residue (EDR)	
raw sample	32.11 ± 0.10
cooked sample[b]	12.64 ± 1.25

[a] Percentage values for samples adjusted to 10% moisture. Mean values ± deviations from three replications. [b] Cooked under pressure (15 psi, 40 min) after being soaked in distilled water for 12 h.

trypsinized bovine erythrocytes (Junqueira and Sgarbieri, 1981). The results were expressed in units of trypsin inhibited (UTI) and in hemagglutination titer (HT), respectively, per gram of sample.

Biological Assays. Diets were prepared containing 10% protein either from casein or from bean, for the nitrogen balance studies, and either 10% or 21.3% protein from various bean cultivars for the protein efficiency ratio and growth assays. The other constituents were soybean oil (8%), mineral mixture (Rogers and Harper, 1965) (4%), vitamin mixture (NBC, 1977/78) (2%), and carbohydrate mixture (25% sucrose:75% starch) (to 100%). A protein-free diet was introduced in all nitrogen balance experiment. Rats of the Wistar strain (40–50-g initial weight) were used for the biological assays. They were caged individually in stainless steel bottom screened cages and maintained at a temperature of 22 ± 1 °C with alternating dark–light period of 12 h. Diet and water were offered ad libitum throughout the experiments.

Protein digestibility, biological value, and net protein utilization were determined by nitrogen balance (Mitchell, 1923; Miller and Bender, 1955; Wolzak et al., 1981). Protein efficiency ratio was done by the AOAC (1980) method. Bioavailable methionine was determined from cooked bean flour supplemented with 1% L-cysteine (on protein basis) as the basic diet and addition of increasing quantities of L-methionine according to Sgarbieri et al. (1979). Calculations were done by using regression equations of body weight gain versus methionine added as percent of dietary protein.

Statistical Analysis. Analysis of variance and the Duncan test (Puri and Muller, 1980) were applied when necessary. Regression equations were constructed by the minimum square method and the Pearson correlation coefficients (r) calculated according to Snedecor (1956).

RESULTS AND DISCUSSION

Table I presents the proximate percent composition of the Carioca 80 cultivar of dry beans. Quantitatively the beans are a good source of proteins (21.8%) and potentially digestible carbohydrate (58.3%) (difference) after the summation of total protein, total lipids, ash, crude fiber, and water (10%) is subtracted from 100%. Characteristically beans of the genus *Phaseolus* are low in total lipids, 1.23% in this cultivar. Table I also shows an estimation of the dietary fiber determined by two different methods in both raw and cooked samples. Both the neutral detergent residue and the pepsin/pancreatin indigestible residue were quite high as determined in the raw sample and was dropped to approximately one-third in the cooked sample. The raw sample in addition to being less digestible contains protease and amylase inhibitors, contributing to the highly indigestible residue. Although one should expect high inhibition of enzyme digestion in the raw sample, due to the presence in beans of digestive enzymes inhibitors, the neutral detergent residue was still higher. We have no explanation for this, but it could be interpreted in terms of low solubility of the raw bean components in the neutral detergent.

Table II. Amino Acids from Bean (Cultivar Carioca 80) and the Casein Utilized as Reference in the Biological Assays

amino acid, g/16 g N	Carioca 80 (raw)	casein
aspartic acid	13.90	8.38
threonine	5.54	5.50
serine	6.35	7.14
glutamic acid	18.29	29.57
proline	3.92	9.32
glycine	5.16	2.17
alanine	5.54	3.43
valine	8.36	7.15
half-cystine	0.27	0.20
methionine	0.95	2.88
methionine + half-cystine	1.22	3.08
isoleucine	5.54	5.21
leucine	9.55	9.89
tyrosine	1.72	5.53
phenylalanine	5.57	5.71
phenylalanine + tyrosine	7.69	11.24
histidine	3.82	3.06
lysine	8.74	7.95
arginine	6.73	4.20
tyrptophan[a]	1.15	1.03

[a] Determined by the colorimetric method of Lunder (1973).

The proximate percent composition of this new cultivar is within the range of variability of other cultivars grown in Brazil. Durigan et al. (1987a) encountered for 12 Brazilian cultivars corrected for 10% water content the following values: protein, 20.7–26.1%; digestible carbohydrate, 51.7–62.7%; ash, 3.3–4.6%; and total lipids, 0.9–1.4%.

According to Hellendoorn (1975) the content of all indigestible carbohydrates of cooked dry beans ranged from 15% to 20% of the whole beans. The insoluble cell wall constituents cellulose, hemicellulose, and lignin constitute about 12–15%, and the remaining 3–5% are soluble material consisting of pectin, arabinogalactan, and galactoside oligosaccharides.

The results reported in Table I for the pepsin/pancreatin residue are in good agreement with those of Hellendoorn (1975) for cooked beans. This residue is not too different from the neutral detergent residue, suggesting that the main components of the indigestible residue should be cellulose, hemicelluloses, lignin, and perhaps some proteins and insoluble minerals.

Trypsin inhibitor activity and lectin activity (hemagglutination) were determined in raw and cooked beans. In bean extract, trypsin inhibitor activity was 1.9×10^4 UTI/g of sample and the hemagglutination was 1.3×10^4 HT/g of sample. Extract from the cooked sample showed a residual inhibitor activity of 950 UTI/g of sample, representing only 5% of the inhibition in the raw sample. No lectin activity was detected in the extract from cooked bean.

The limiting essential amino acid in *Phaseolus* is methionine. Total sulfur-containing amino acids in the cultivar Carioca 80 (Table II) are less than half the amount found in casein.

Table III shows the content of total methionine in the cultivar Carioca 80 in raw and cooked samples, determined by three different procedures, as well as the content of bioavailable methionine as described by the method of Sgarbieri et al. (1979). One can observe considerable variability in the determination of total methionine depending on the method utilized.

Table III. Determination of Total Bean (Carioca 80) Methionine by Various Methods

method	g Met/16 g N[a]	
	raw bean	cooked[b] bean
acid hydrol, ion exch	0.95 ± 0.04	0.98 ± 0.03
enzymatic hydrol, sodium nitroprusside colorimetric reactn of hydrolysate	1.42 ± 0.01	1.55 ± 0.07
BrCN reactn, GC of byproduct, CH_3SCN	1.05 ± 0.07	1.48 ± 0.18
rat growth assay (bioavailable Met)[c]		0.84

[a] Results are means ± deviations of three replications. [b] Cooked under pressure (15 psi, 40 min) after soaking in distilled water for 12 h. [c] Calculated by the equation $y = 18.11X + 15.07$ ($r = 0.96$, $p < 0.001$).

Acid hydrolysis of the bean flour followed by ion-exchange separation and ninhydrin reaction in the amino acid analyzer gave the lowest recovery of methionine. Enzymatic hydrolysis followed by colorimetric reaction in the soluble hydrolysate gave the highest recovery, which was not too different, in the cooked sample, from the result obtained by reaction of the bean flour with BrCN followed by GLC of the CH_3SCN in the soluble fraction. Methionine determined in the acid hydrolysate of 12 cultivars by ion-exchange chromatography was constant at 1.0–1.1 g of methionine/100 g of amino acids recovered while the BrCN reaction followed by gas–liquid chromatography of the CH_3SCN formed furnished values varying from 0.96 to 1.99 g of methionine/16 g of N (Durigan et al., 1987a). These values are comparable with the ones of Table III found for the cultivar Carioca 80.

The bioavailable methionine of 0.84 g/100 g of crude protein (Table III) is considered high when compared with that of other Brazilian cultivars. Durigan et al. (1987a) encountered in 12 cultivars methionine bioavailability ranging from 0.23 to 0.77 g of methionine/100 g of crude protein (mean value 0.51). The percent bioavailability for the 12 cultivars determined by Durigan et al. (1987a) ranged from 29.3% to 40.2% (mean value 33.4%) using the BrCN reaction for quantification of methionine. By use of the same calculation for the new cultivar (Carioca 80) methionine bioavailability was 56.8%, higher than that of any other Brazilian cultivar and also higher than the value of 50% reported by Evans and Bauer (1978) for navy bean, using the metabolic balance technique.

The immediate consequence of higher bioavailability of methionine in this new bean cultivar (Carioca 80) is higher biological value and protein utilization by the rat (Table IV). The true biological value of 78.5% (diet B) found for the protein of this cultivar was substantially higher than the values (38.3–58.9%) encountered for four other Brazilian cultivars (Sgarbieri et al., 1979). The cultivar Carioca, which served as the genetic basis for the development of Carioca 80, showed a methionine bioavailability of 29.3% and a protein biological value of 39.4%. Referring again to Table IV, diet C with 0.3% methionine added to the bean flour, on a protein basis, showed a protein biological value equal or superior to that of casein. Adding 0.6% methionine did not further improve the protein biological value in the bean flour.

The influence of removing the tegument (seed peel) and discarding the soaking water, prior to cooking, in the nutritive value of Carioca 80 protein is presented in Table V. By comparing diets E and F, one sees that discarding the maceration (soaking) water did not improve significantly protein digestibility but did improve protein biological value. The difference between 81.0 and

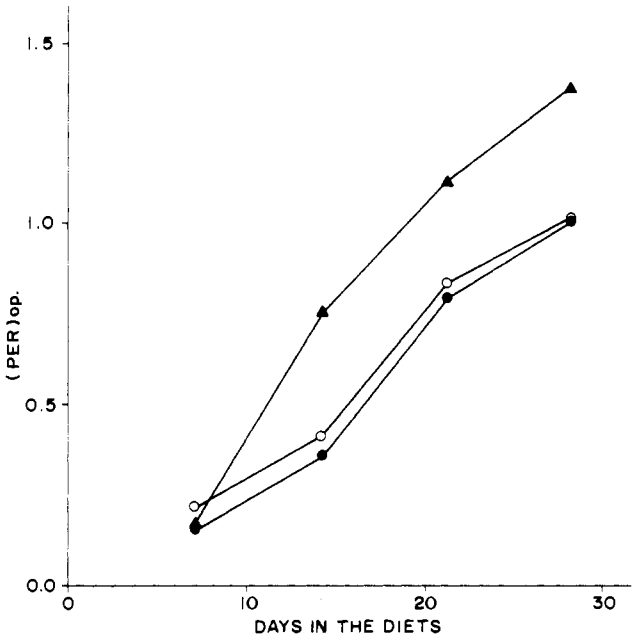

Figure 1. Body weight gain per protein consumed in grams [$(PER)_{op}$] as a function of days on bean diets (21% protein) of weanling Wistar rats. Cultivars: Carioca 80 (▲); Carioca (●); Aeté 3 (○). Each point represents the mean value for a group of eight rats.

71.9% was statistically significant ($p < 0.05$). Increasing soaking time from 12 to 48 h (diets E and G) significantly decreased digestibility and biological value of the proteins ($p < 0.05$) when the maceration water was discarded. Removing the seed tegument and discarding the soaking water (48 h of soaking) prior to cooking resulted in the highest improvement in both digestibility and biological values (diet H). A comparison between diets G and H indicates that the seed coat contributes to decreased digestibility and biological value of bean protein. The results suggest that both the tegument and the water after soaking should contain substances that interfere with the digestibility and the biological value of bean protein. Similar observations have been reported by Sgarbieri et al. (1982) and Sgarbieri and Garruti (1986). Although the interfering factors have not been identified, the bean tegument contains most of the phenolic compounds of the seeds, which may have an influence on protein digestibility and utilization. On the other hand the soaking water may contain, in addition to phenolics, phytic acid and protease inhibitors, which are resistant to heat denaturation (Sgarbieri et al., 1982).

On the basis of methionine bioavailability, protein digestibility, biological value, and NPU (Tables III and IV), it was expected that PER for the Carioca 80 protein would be considerably higher than for other commercial cultivars. However, when Carioca 80 was compared with cultivars Carioca and Aeté 3, with lower methionine bioavailability and lower biological value at 10% dietary protein, the PER for Carioca 80 was not superior to those for the other two cultivars. The PER values for the three bean cultivars were below 1.0. However, when bean entered in the composition of the diet to furnish 21% protein, which was essentially bean flour supplemented with minerals and vitamins (Figure 1), the PER values of the three cultivars of bean were low in the first week but increased steadily throughout the experimental period. At the end of the experiment (28 days) the cultivar Carioca 80 showed a PER about 40% higher than those of the other two cultivars (Figure 1).

Table IV. Digestibility, Biological Value, and NPU for the Bean (Cultivar Carioca 80) Protein with and without Methionine Supplementation, Compared with Casein

determination[a]	treatment[b] A	B	C	D
apparent digestibility, %	92.3 ± 1.08[a]	64.1 ± 2.19[b]	65.2 ± 4.61	64.2 ± 1.68[b]
true digestibility, %	93.7 ± 1.18[a]	66.2 ± 1.99[b]	67.1 ± 4.21[b]	66.1 ± 1.83[b]
apparent biological value, %	85.2 ± 3.06[a]	68.5 ± 4.10[b]	83.3 ± 2.50[a]	83.7 ± 2.50[a]
true biological value, %	89.4 ± 3.21[a]	78.5 ± 4.67[b]	91.7 ± 2.64[b]	92.4 ± 2.88[a]
apparent, NPU, %	78.6 ± 3.24[a]	43.9 ± 3.70[b]	54.3 ± 1.85[c]	54.9 ± 2.55[c]
true NPU, %	83.7 ± 3.34[a]	52.2 ± 4.50[b]	61.4 ± 2.09[c]	61.1 ± 2.83[c]

[a] Data are mean values of five rats/treatment. (a–c) different letters in horizontal lines represent results statistically different at $p < 0.05$. [b] Key: A = diet containing 10% casein as sole source of protein; B = diet containing 10% bean protein as sole source of protein; C = diet containing 10% bean protein + 0.3 g of methionine/100 g of protein; D = diet containing 10% bean protein + 0.6 g of methionine/100 g of protein.

Table V. Influence of Removing the Tegument (Peel) and Discarding the Maceration Water Prior to Cooking on Digestibility and Biological Value of the Carioca 80 Bean Protein (Bean Used To Provide 10% Protein in the Diet)

determination[a]	treatment[b] E	F	G	H
nitrogen ingested, g	0.91 ± 0.06	0.81 ± 0.11	0.71 ± 0.08	0.97 ± 0.10
fecal nitrogen, g	0.29 ± 0.02	0.27 ± 0.05	0.26 ± 0.03	0.24 ± 0.02
urine nitrogen, g	0.17 ± 0.02	0.20 ± 0.05	0.16 ± 0.02	0.15 ± 0.02
nitrogen absorbed, g	0.62 ± 0.02	0.54 ± 0.05	0.45 ± 0.02	0.73 ± 0.03
nitrogen retained, g	0.45 ± 0.02	0.34 ± 0.05	0.29 ± 0.02	0.58 ± 0.03
true digestibility, %	71.43 ± 3.43[a]	70.37 ± 2.87[a]	67.61 ± 2.11[b]	78.35 ± 3.04[c]
true biological value, %	81.00 ± 2.90[a]	71.92 ± 7.00[b]	76.30 ± 0.05[c]	85.53 ± 0.94[c]

[a] Results are mean values ± deviations, five rats per treatment. (a–d) different letters in horizontal lines indicate statistically different results ($p < 0.05$). [b] Key: E = diet prepared with bean macerated for 12 h and cooked with the tegument after the maceration water was discarded; F = diet prepared with bean macerated for 12 h and cooked with the tegument in the same maceration water; G = diet prepared with bean macerated for 48 h and cooked with the tegument after the maceration water was discarded; H = diet prepared with bean macerated for 48 h and cooked without the seed tegument after the maceration water was discarded.

The data presented in this paper for the new cultivar Carioca 80, when contrasted with reported data for other bean cultivars, permitted the following conclusions: (1) Proximate percent composition and amino acid profile are quite similar in Carioca 80 compared to other studied cultivars. (2) Methionine bioavailability is considerably higher in Carioca 80 when compared to other Brazilian cultivars. (3) As a consequence of the high methionine bioavailability and the higher protein digestibility, Carioca 80 had a much higher protein biological value and NPU. (4) When the PER value for Carioca 80 at a 10% bean protein level was compared with Carioca and Aeté 3, they did not differ significantly; however, when the diets were prepared with 21% bean protein, instead of 10%, PER increased considerably with time on the diet. After 4 weeks it was 40% higher for Carioca 80, compared with Carioca and Aeté 3. This could have resulted from two factors: (1) The 10% dietary protein seems to be insufficient to cause appreciable growth in the rat and it shows no difference in growth as a result of higher methionine bioavailability; however, doubling the protein in the diet significantly increased growth and PER for Carioca 80, as a result of higher bioavailability of methionine. (2) The fact that PER increased considerably with feeding time seems to indicate adaptation of the rats to the bean diets. This phenomenon has already been reported for other cultivars of beans (Durigan et al., 1987b).

The results suggest that the standard procedure (AOAC, 1980) for determination of PER with 10% dietary protein may not be an appropriate method for detecting differences in nutritive value of bean proteins.

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