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Protein Quality of Vegetable Proteins As Determined by Traditional Biological Methods and Rapid Chemical Assays

Arlene Wolzak, Luiz G. Elías, and Ricardo Bressani*

Traditional biological assays were compared with chemical estimates of protein quality by using different vegetable proteins. The comparability and reproducibility of protein efficiency ratio (PER), net protein ratio (NPR), and in vivo protein digestibility were tested in two experiments at different times. A highly significant correlation was found between PER and NPR in both experiments, although a higher correlation was observed in the second, in which a smaller and more homogeneous group of samples was tested. The PER showed the best reproducibility. Amino acid scores, essential amino acid indexes, and C-PER values were calculated. PER correlated better with chemical parameters than with NPR. The amino acid score, though an imperfect indicator, still seems to be the best of the chemical parameters studied. C-PER values showed a highly significant correlation with PER for the complete group of samples ($r = 0.871$; $n = 33$), although they overestimated the protein quality of leguminous seeds and processed samples and underestimated that of mixtures supplemented with animal protein.

The nutritional quality of a protein is determined by the quantity, availability, and proportions of the essential amino acids comprising it and the presence, for optimum utilization, of sufficient nonessential amino acids. Bioassays measure the efficiency of the biological utilization of dietary proteins as sources of the essential amino acids under a set of standardized conditions (Lachance et al., 1977).

Many biological methods based on the effects of the quality and amount of dietary protein on growth performance in young animals have been proposed for evaluating protein quality. Among these methods, the protein efficiency ratio (PER), based on weight changes of growing rats, is perhaps the most widely used. This method has been severely criticized by several authors (Pellett, 1978; Steinke, 1977). One of its shortcomings is that no consideration is given to the requirements of protein for maintenance. To overcome this objection, the inclusion of a group of animals consuming a nonprotein diet for a similar period of time was proposed and the procedure is called net protein ratio (NPR; Bender and Doell, 1957).

Biological assays are expensive and time-consuming and require considerable amounts of samples which are not always available. As a result, chemical methods based on amino acid composition of the proteins and enzymatic assays for the measurement of protein quality and digestibility have been devised. Important examples of such assays are the amino acid score (Mitchell and Block, 1946) and the essential amino acid index (Oser, 1951). Recently, the C-PER method was developed (Satterlee et al., 1977). This method corresponds to a PER value derived from the essential amino acid profile and the protein digestibility as determined by a multienzyme in vitro assay (Hsu et al., 1977; Satterlee et al., (1979). These assays require small amounts of samples and provide results on protein quality in shorter periods of time than biological assays.

The purpose of this study was to compare the results of two traditional biological assays, the PER and the NPR, with chemical estimates of protein quality such as amino acid score, essential amino acid index, and C-PER using a group of vegetable proteins widely consumed in developing countries.

MATERIALS AND METHODS

Samples and Sample Preparation. Protein samples were selected to include a set of vegetable proteins covering a wide range of protein quality. The samples used were commercial and laboratory-prepared plant proteins such as cereal grains, leguminous seeds, oilseeds and byproducts, and mixtures of cereal grains and leguminous seeds alone and supplemented with powdered skim milk or meat meal. ANRC casein was used as reference protein.

Leguminous seeds were prepared according to the technique previously described (Elías et al., 1976). Immature corn kernels were dried ($T = 40$ °C) and ground. Sesame seeds (*Sesamum indicum*) were pressed in a disk mill, extracted with hexane, and ground in a hammer mill. Commercial samples included soybean meal, cottonseed meal, white wheat flour, and the corn and bean flours used in the mixtures. The rest of the samples were ground in a hammer mill to pass a 60-mesh screen.

Chemical Assays. The nitrogen content of all the samples was determined by the macro-Kjeldahl method (AOAC, 1970). The crude protein was calculated by using the appropriate factors (FAO/WHO, 1973).

In Vitro Digestibility Experiments. The in vitro digestibility of the samples was assessed by measuring the extent to which the pH of the protein suspension dropped

Center for Higher Studies in Nutrition and Food Sciences (CESNA), University of San Carlos de Guatemala/Institute of Nutrition of Central America and Panama (INCAP), Guatemala, Central America (A.W.), and Division of Agricultural and Food Sciences, Institute of Nutrition of Central America and Panama (INCAP), Guatemala City, Guatemala, Central America (L.G.E. and R.B.).

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Table I. PER, NPR, and Apparent Protein Digestibility of Proteins in the First Experiment

protein sample	protein in diet, %	PER[a]	NPR[a]	apparent in vivo protein digestibility[a]
casein	7.4	2.09 ± 0.28	4.11 ± 0.32	90.7 ± 1.2
casein	9.3	2.62 ± 0.30	4.44 ± 0.25	
common corn	8.5	1.10 ± 0.18	2.72 ± 0.29	83.0 ± 3.3
immature corn	8.9	1.76 ± 0.15	3.70 ± 0.26	78.6 ± 2.4
cornflakes	6.7	−0.57 ± 0.29	2.72 ± 0.44	72.0 ± 4.7
cornmeal	6.9	0.16 ± 0.34	3.17 ± 0.75	86.5 ± 1.9
opaque-2 corn	6.5	1.96 ± 0.54	3.94 ± 0.48	80.3 ± 2.3
white sorghum	7.4	0.76 ± 0.34	2.93 ± 0.40	80.6 ± 3.3
red sorghum	7.0	0.78 ± 0.24	3.05 ± 0.52	77.4 ± 6.3
rice	6.7	1.78 ± 0.41	4.05 ± 0.60	86.0 ± 2.8
white wheat flour	9.2	0.64 ± 0.10	2.12 ± 0.39	90.7 ± 2.5
wheat	9.5	1.34 ± 0.12	3.00 ± 0.33	81.6 ± 2.7
black beans, 20 min[b]	9.1	−0.10 ± 0.28	1.89 ± 0.61	73.2 ± 4.0
white beans, 20 min	9.5	0.74 ± 0.22	2.33 ± 0.31	74.1 ± 7.0
red beans, 20 min	9.0	−0.02 ± 0.19	2.22 ± 0.31	71.2 ± 4.5
cowpea, 20 min	9.4	0.99 ± 0.39	2.51 ± 0.42	80.0 ± 2.0
pigeon pea, 20 min	9.5	1.19 ± 0.25	2.41 ± 0.33	76.4 ± 3.6
soybean, 20 min	9.1	2.43 ± 0.39	4.44 ± 0.63	80.5 ± 3.0
soybean flour	8.8	2.50 ± 0.31	3.94 ± 0.59	83.0 ± 1.7
cottonseed flour	8.2	1.41 ± 0.23	3.00 ± 0.41	76.9 ± 4.4
sesame seed flour	9.0	0.99 ± 0.27	2.50 ± 0.42	84.4 ± 1.9
corn-black beans, 87:13[c]	9.1	2.07 ± 0.16	3.02 ± 0.27	82.6 ± 2.2
corn-black beans, 70:30	8.5	2.31 ± 0.10	3.81 ± 0.33	79.2 ± 2.1
rice-black beans, 95:5	7.2	2.35 ± 0.49	4.01 ± 0.53	82.3 ± 2.4
rice-black beans, 80:20	9.1	2.38 ± 0.25	3.58 ± 0.46	80.0 ± 2.1
pigeon pea-immature corn, 25:75	9.3	2.00 ± 0.19	3.37 ± 0.37	76.9 ± 2.5
corn-black beans, 87:13, + 5% skim milk	9.6	2.39 ± 0.16	3.46 ± 0.23	82.3 ± 1.9
corn-black beans, 87:13, + 10% meat meal	9.2	2.93 ± 0.21	4.27 ± 0.27	86.1 ± 1.2
rice-black beans, 95:5, + 5% skim milk	6.9	2.90 ± 0.34	5.23 ± 0.33	81.5 ± 1.4
rice-black beans, 95:5, + 10% meat meal	9.1	3.29 ± 0.20	5.34 ± 0.30	86.3 ± 1.7
corn-soybean, 70:30	9.1	2.54 ± 0.19	4.31 ± 0.32	79.5 ± 2.5

[a] Mean ± SD. [b] Indicates cooking time. [c] Mixtures by weight.

Table II. PER, NPR, and Apparent Protein Digestibility of Proteins in the Second Experiment

protein sample	protein in diet, %	PER[a]	NPR[a]	apparent protein digestibility
casein	7.4	2.09 ± 0.28	4.11 ± 0.32	87.7 ± 3.1
casein	9.2	2.57 ± 0.22	4.34 ± 0.26	91.4 ± 0.6
rice[b]	6.5	1.99 ± 0.20	3.68 ± 0.36	81.9 ± 3.5
white wheat flour[b]	8.7	0.98 ± 0.22	2.09 ± 0.33	89.4 ± 1.0
pigeon pea, 20 min[b]	9.2	1.21 ± 0.30	2.23 ± 0.51	73.7 ± 4.2
red bean, 20 min[b]	9.1	0.16 ± 0.17	1.61 ± 0.44	69.0 ± 7.5
white bean, 20 min[b]	9.0	1.21 ± 0.36	2.48 ± 0.40	71.4 ± 4.1
black bean, 20 min[b]	9.1	0.25 ± 0.28	1.62 ± 0.43	72.5 ± 7.5
cowpea, 20 min[b]	9.1	1.09 ± 0.23	2.10 ± 0.20	76.0 ± 2.1
cowpea, 10 min[c]	9.0	1.32 ± 0.22	2.27 ± 0.36	74.3 ± 2.5
black bean, 30 min[c]	9.0	0.29 ± 0.36	1.47 ± 0.45	68.2 ± 3.2
white bean, 30 min[c]	8.7	0.81 ± 0.28	1.90 ± 0.46	75.4 ± 5.2
soybean, 20 min[b]	8.9	2.48 ± 0.20	3.86 ± 0.45	78.0 ± 1.7
soybean flour[b]	8.7	2.72 ± 0.36	3.97 ± 0.47	79.7 ± 2.9
cottonseed flour	8.7	1.38 ± 0.30	2.50 ± 0.43	73.4 ± 4.0

[a] Mean ± SD. [b] Same sample used in the first experiment. [c] Same sample as in the first experiment but processed differently as indicated by the cooking time.

Table III. Correlation Coefficients between Traditional Biological Assays

	n	r
correlation between methods		
PER vs. NPR (first expt)	240	0.801[a]
PER vs. NPR (second expt)	120	0.919[a]
reproducibility of methods		
NPR (first expt) vs. NPR (second expt)	88	0.835[a]
PER (first expt) vs. PER (second expt)	88	0.915[a]
apparent protein digestibility	88	0.744[a]

[a] $P < 1\%$.

when treated with a multienzyme system including trypsin, chymotrypsin, and aminopeptidase as described by Hsu et al. (1977) and modified by Satterlee et al. (1979) by adding a fourth enzyme, *Streptomyces griseus* protease, to complete proteolysis. These results have been previously reported (Wolzak et al., 1981).

In Vivo Experiments. Male and female weanling rats of the Wistar strain from the INCAP colony, 21–23 days of age, were used as experimental animals. The groups were formed by eight rats, four males and four females. The rats were housed in individual, all-wire screen cages and were allowed free access to food and water. The basal diet consisted of the following: cornstarch, 90%; mineral mixture (Hegsted et al., 1941), 4%; cottonseed oil, 5%; cod liver oil, 1%; supplemented with 5 mL of vitamin solution (Manna and Hauge, 1953). Protein test diets were made by replacing cornstarch in the basal diet with each protein

Table IV. Chemical and Biological Parameters of Protein Quality

sample	PER	NPR	in vitro protein digestibility	amino acid score[a] net	amino acid score[a] corrected[b]	essential AA index net	essential AA index corrected[b]	C-PER	diff[c,d]
casein	2.62	4.44	87.1	92.0 S	80.1	99.0	86.2	2.50	0.12
common corn	1.10	2.72	82.0	48.5 L	39.8	84.9	69.6	1.23	−0.13
immature corn	1.76	3.70	78.4	62.0 L	48.6	88.8	69.6	1.50	0.26
cornflakes	−0.57	2.72	67.2	34.5 L	23.2	80.1	53.8	0.38	−0.94
cornmeal	0.16	3.17	84.0	52.3 L	43.9	85.5	71.8	1.37	−1.21
opaque-2 corn	1.96	3.94	80.3	76.5 L	61.4	90.2	72.4	1.87	0.09
white sorghum	0.76	2.93	79.5	36.7 L	29.2	83.0	66.0	0.66	0.10
red sorghum	0.78	3.05	77.5	36.7 L	28.4	83.0	64.3	0.63	0.15
rice	1.78	4.05	84.8	69.1 L	58.6	93.8	79.5	1.98	−0.20
white wheat flour	0.64	2.12	85.8	41.4 L	35.5	85.2	73.1	1.01	−0.37
wheat	1.34	3.00	83.7	55.8 L	46.7	88.1	73.7	1.59	−0.25
black bean, 20 min[e]	0.25	1.62	72.7	54.3 S	39.5	91.6	66.6	1.30	−1.05
white beans, 20 min[e]	1.21	2.48	75.3	54.3 S	40.9	91.6	69.0	1.37	−0.16
red beans, 20 min[e]	0.16	1.61	71.1	54.3 S	38.6	91.6	65.1	1.27	−1.11
cowpea, 20 min[e]	1.09	2.10	75.3	64.6 S	48.6	91.8	69.1	1.67	−0.58
pigeon pea, 20 min[e]	1.21	2.23	73.0	42.9 S	31.3	73.8	53.9	0.72	0.49
cowpea, 10 min[e]	1.32	2.27	75.3	64.6 S	48.6	91.8	69.1	1.67	−0.35
black beans, 30 min[e]	0.29	1.47	71.4	54.3 S	38.8	91.6	65.4	0.68	−0.39
white beans, 30 min[e]	0.81	1.90	73.7	54.3 S	40.0	91.6	67.5	1.33	−0.52
soybeans, 20 min	2.43	4.44	85.5	81.1 S	69.3	97.4	83.3	2.31	0.12
soybean flour	2.50	3.94	85.8	96.6 S	82.9	99.6	85.4	2.57	−0.07
cottonseed flour	1.41	3.00	77.6	75.3 S	58.4	85.7	66.5	1.05	0.36
sesame seed flour	0.99	2.50	80.5	49.6 L	39.9	87.8	70.7	0.93	0.06
corn-beans, 87:13	2.07	3.02	80.3	71.6 L	57.5	89.4	71.8	1.81	0.26
corn-beans, 70:30	2.31	3.81	82.4	75.4 S	62.1	92.2	76.0	1.97	0.34
rice-beans, 95:5	2.35	4.01	83.3	77.1 L	64.2	95.4	79.5	2.18	0.17
rice-beans, 80:20	2.38	3.58	82.6	86.6 S	71.5	96.6	79.8	2.27	0.11
corn-beans, 87:13, + 5% skim milk	2.39	3.46	81.9	82.0 L	67.2	94.1	77.1	2.07	0.32
corn-beans, 87:13, + 10% meat meal	2.93	4.27	82.4	95.7 Thr	78.8	98.7	81.3	2.29	0.64
rice-beans, 95:5, + 5% skim milk	2.90	5.23	84.4	88.5 L	74.7	97.7	82.4	2.17	0.73
rice-beans, 95:5, + 10% meat meal	3.29	5.34	85.2	95.0 Thr	80.9	99.4	84.7	2.49	0.80
pigeon pea-immature corn, 25:75	2.00	3.37	79.3	60.0 Trp	47.6	88.0	69.8	1.56	0.44
corn-soybean, 70:30	2.54	4.31	82.9	87.7 S	72.7	98.0	81.2	2.27	0.27

[a] Limiting essential amino acid: L = lysine; S = sulfur amino acids; Trp = tryptophan; Thr = threonine. [b] Net value corrected by in vitro protein digestibility. [c] PER − C-PER. [d] Mean difference = −0.045; SD = 0.514; SE = 0.090. [e] Biological parameters obtained in the second experiment.

source at a level to provide 9% protein when possible, or 7% in the case of samples of low protein content.

Body weights and food consumption were recorded after 10 days and thereafter on a weekly basis for a total period of 28 days. An additional group of eight rats was fed a nonprotein diet during the first 10 days of the assay, and the average weight loss was used in the calculation of the net protein ratio (NPR). The weight changes and protein consumption over the 28-day period of time were used to estimate protein efficiency ratios. The feces of each rat were collected during the last week of the experiment, air-dried, weighed, and analyzed for N to calculate apparent protein digestibility.

A group of samples was assayed at two different times to test for the reproducibility of the biological methods used.

The comparability and reproducibility of the protein apparent digestibility and of the different protein quality parameters were evaluated by simple regression analysis.

Amino Acid Data. The essential amino acid content used in the calculation of chemical parameters of protein quality was obtained from food composition tables (FAO, 1970; Orr and Watt, 1957). The FAO/WHO (1973) amino acid pattern was used as reference in the calculation of amino acid scores and essential amino acid indexes. C-PER values were obtained as described by Hsu et al. (1978).

RESULTS AND DISCUSSION

The PER, NPR, and apparent protein digestibility values for the first and second assays, as well as the protein content of the diets, are shown in Tables I and II. It can be seen from these values that a wide interval of protein quality was covered in the first assay. PER values ranged from 3.29 to −0.57, while NPR went from 5.34 to 1.89. Both methods ranked the rice–bean (95:5) plus 10% meat meal as the highest quality protein. However, the PER assay ranked cornflakes as the poorest protein source, while the lowest NPR value was obtained for black beans. In general, it was found that NPR values for leguminous seeds were lower than the corresponding PER values.

The correlation coefficients between PER and NPR values for both experiments, as well as between values obtained at different times with the same samples, are shown in Table III. The correlation between PER and NPR values was highly significant for both experiments ($r = 0.801$ for the first experiment and $r = 0.919$ for the second) and similar to others previously reported (Lachance et al., 1977). The correlation coefficient of the second experiment was statistically higher than that of the first assay. This can be attributed to the fact that a more homogeneous group of samples was assayed in the second experiment; it also confirms previous observations that the correlation between bioassays depends, to a certain extent, on the type of samples tested.

When correlation coefficients between PER and NPR values were calculated by groups of samples, it was found that the cereal grain group showed a value of 0.777 ($n = 11$) and that, when corn flakes were excluded, the value rose to 0.922. Thermal processing affects protein quality, rendering basic amino acids, and especially lysine, unavailable. This explains its different behavior from the other nonprocessed cereal grains. It has been suggested that different amino acid deficiencies do not necessarily result in equivalent responses; thus, some authors claim that NPR overestimates lysine-deficient proteins, while PER strongly penalizes them (Bodwell, 1979; McLaughlan and Keith, 1975). This observation was found valid in our study.

Table V. Correlation Coefficients between Biological and Chemical Parameters of Protein Quality

comparison	excluding leguminous seeds ($n = 25$)	including leguminous seeds ($n = 33$)
PER vs. AA score	0.916[b]	0.884[b]
PER vs. cor AA score[a]	0.919[b]	0.906[b]
PER vs. EAA index	0.913[b]	0.683[b]
PER vs. cor EAA index[a]	0.849[b]	0.819[b]
NPR vs. AA score	0.804[b]	0.744[b]
NPR vs. cor AA score[a]	0.813[b]	0.799[b]
NPR vs. EAA index	0.838[b]	0.553[b]
NPR vs. cor EAA index[a]	0.748[b]	0.767[b]
AA score vs. EAA index	0.937[b]	0.859[b]
AA score vs. EAA index (cor)	0.894[b]	0.899[b]
C-PER vs. score	0.934[b]	0.929[b]
C-PER vs. cor AA score	0.954[b]	0.944[b]
C-PER vs. EAA index	0.960[b]	0.872[b]
C-PER vs. cor EAA index	0.932[b]	0.911[b]

[a] Raw value corrected by in vitro protein digestibility. [b] $P < 1\%$.

The correlation coefficient between PER and NPR values for the group of 10 leguminous seeds assayed in the second experiment was 0.97, and for the mixtures ($n = 10$) it was 0.906. Similar values have been previously reported by Lachance et al. (1977), who found an $r = 0.89$ for a group of 18 unprocessed cereal grains and of 0.96 for a group of 21 leguminous seeds. This further confirms the observation that the type of sample and, more specifically, the limiting amino acid will influence the predictive capacity of different protein quality bioassays.

The reproducibility of the PER method was better than that of the NPR as shown by the correlation coefficients between experiments for each method (0.915 for PER; 0.835 for NPR). A highly significant reproducibility between apparent digestibility values was also found, although the values in the second experiment were consistently lower.

Table IV presents chemical parameters of protein quality for the samples tested, as well as PER and NPR values for comparison. A major criticism of the use of chemical parameters to estimate protein quality is that not all the amino acids determined by chemical analysis are available to the living organism, which results in higher estimates of protein quality, especially in samples of low digestibility. To overcome this, it has been suggested that raw chemical scores should be corrected by the in vitro digestibility of the samples. These corrected values are also presented in Table IV. From the C-PER values and their differences from in vivo PER, it can be seen that relatively good estimates were obtained for nonprocessed cereal grains and oilseeds as well as for cereal grain–leguminous seed mixtures. This suggests that the C-PER method is sensitive to protein complementation. However, the C-PER values overestimated the protein quality of leguminous seeds and processed samples and underestimated the quality of mixtures containing animal protein supplements. In processed samples, the in vitro digestibility is usually higher than the in vivo value, and this results in an overestimation of the protein quality of these types of samples. Cornmeal and other low protein content

Table VI. Correlation Coefficients between PER and C-PER Values

sample	n	r
all samples	33	0.871[c]
cereal grains[a]	11	0.864[c]
cereal grains excluding cornflakes and cornmeal	9	0.946[c]
leguminous seeds[a]	9	0.741[c]
leguminous seeds[b]	8	0.335[d]
oilseeds[a]	5	0.981[c]
oilseeds[b]	4	0.984[c]
cereal grains and oilseeds[a]	15	0.881[c]
cereal grains and oilseeds[b]	14	0.857[c]
cereal grains and oilseeds[a] excluding cornflakes, cornmeal, and wheat flour	12	0.965[c]
cereal grains and oilseeds[a] excluding cornflakes and cornmeal	13	0.953[c]
mixtures[a]	11	0.784[c]
mixtures[b]	10	0.829[c]
cereal grains and mixtures[a]	21	0.916[c]
cereal grains and mixtures[a] excluding cornflakes and cornmeal	19	0.935[c]
cereal grains, mixtures, and oilseeds[a]	25	0.907[c]
cereal grains, mixtures, and oilseeds[a] excluding cornflakes and cornmeal	23	0.931[c]

[a] Including casein in the calculation. [b] Not including casein in the calculation. [c] $P < 1\%$. [d] Not significant.

samples are difficult to evaluate by traditional biological assays (Hsu et al., 1978), and the difference between C-PER and PER values could be due to this fact. For animal protein supplemented mixtures, the underestimation which occurs has been related to a lower in vitro digestibility than the in vivo value which influences the C-PER estimate (Hsu et al., 1978). From these results, it seems that the C-PER method cannot be applied to all kinds of samples. For leguminous seeds, the difference between C-PER and PER values is related to the difficulty of predicting protein quality of samples with relatively good essential amino acid balance but with low in vivo digestibility.

The correlation coefficients between biological and chemical parameters, including all the samples or excluding leguminous seeds, are shown in Table V. It was found that PER values correlated better than the NPR with the amino acid scores and essential amino acid indexes. Both PER and NPR correlated better with the amino acid scores than with the essential amino acid indexes. These results confirm that even though the amino acid score can be considered as an imperfect indicator of protein quality, it still is the best of those based on amino acid composition (Woodham and Deans, 1977). This is due to the fact that protein quality as determined by biological procedures depends only on the limiting essential amino acid. For the essential amino acid index, the importance of the first limiting amino acid deficiency is diluted, which makes it less sensitive to changes in protein quality.

It can be seen that, in general, excluding the leguminous seeds from the calculations improves the correlation between biological and chemical parameters. When chemical values of protein quality are corrected by protein digestibility, a slight improvement is observed in the correlation coefficient between them and the PER when all samples are included, but this does not occur when leguminous seeds have been excluded from the calculation. Several authors have questioned the usefulness of correcting chemical parameters by the sample's protein digestibility (Bodwell, 1977, 1981). From a practical point of view, it can be said that the benefit of performing an in vitro assay depends on the sample being evaluated (Satterlee et al., 1981). For leguminous seeds and thermally processed samples, although a relatively good essential amino acid profile may be determined chemically, their digestibility and availability is low; thus, a correction factor is required.

Table VI shows the correlation coefficients between PER and C-PER values for all samples together and by groups. The overall correlation was highly significant ($r = 0.871$; $n = 33$). Group analysis showed that a high correlation exists in cereal grains, oilseeds, and mixtures individually and also when grouped together. The correlation coefficients rise when cornflakes, cornmeal, and white wheat flour are excluded. For leguminous seeds alone, the correlation coefficient between PER and C-PER was nonsignificant, which suggests that the method as such is not adequate to predict the protein quality of these samples.

The need for more and better sources of protein to feed the growing population of the world has motivated much research toward the development of rapid protein quality assays. The difficulty of finding a single rapid method capable of accurately predicting the protein quality of all types of samples must be stressed. The amino acid score should be preferred over the essential amino acid index as a fast estimator of protein quality. In the case of leguminous seeds, PER and NPR gave essentially the same results; NPR is recommended because it is completed in 10 days and requires less sample. For unprocessed cereal grains, oilseeds, and mixtures, C-PER values give adequate estimations of protein quality. The rapid protein evaluation of samples containing animal proteins and processed vegetable proteins requires more research.

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Composition and Digestibility of Albumin, Globulins, and Glutelins from *Phaseolus vulgaris*

Ursula M. L. Marquez and Franco M. Lajolo*

Fifteen Brazilian varieties of *Phaseolus vulgaris* were tested for digestibility in vitro, trypsin inhibitor, and protein content. Four varieties with extreme digestibility values were assayed in rats and showed similar digestibilities in vivo. The protein from the Carioca variety fractionated for detailed studies yielded the following: albumin, 31.5% (richest in sulfur amino acids and trypsin inhibitors); globulin G_1, 38.5%; globulin G_2, 13.8%; glutelin, 22.4%. The in vitro digestibilities of the unheated globulins and glutelins were low but improved by heating. The albumins were well digested in the raw state but after heating digestibility dropped; the effect was pH dependent. The residue left after digestion of autoclaved albumin contained peptides with molecular weights of 14 000 and 20 000. Evidences on a relatively heat stable trypsin inhibitor in the albumin fraction are presented. The extent of digestion of the four fractions was tested by using either trypsin, pancreatin, or pepsin–pancreatin.

Beans (*Phaseolus vulgaris*) are an important source of protein in Brazil where they are usually consumed together with rice. However, several biochemical problems limit the optimal biological utilization of the amino acids (Kakade, 1974; Bressani and Elias, 1979).

The low digestibility of bean protein has been documented, but the reasons for it are not well understood and are probably due to a combination of factors. Improper storage at high relative humidities is known to increase cooking time and to reduce the protein digestibility and the biological utilization of bean amino acids (Molina et al., 1975; Antunes and Sgarbieri, 1979). Excessive heating to inactivate antinutritional factors is also prejudicial to the digestibility and amino acid availability; processes such as dry roasting and extrusion cooking are better for maintaining the biological value of beans (Molina et al., 1975; Yadav and Liener, 1978). Antiphysiological factors such as hemagglutinins and trypsin inhibitors are inactivated by proper heat treatment and can probably be excluded as a cause. The exception may be the heat-stable protease (enzyme) inhibitors which seem to be phenolic in nature and present in the seed coat of some colored beans (Elias et al., 1979).

Protein-complexing substances such as tannins appear to be partly implicated. They can either be extracted in the cooking water or migrate to the center of the cotyledon, thus reducing digestibility directly by reacting with the proteins or indirectly by inactivating digestive enzymes. The low biological value of the cooking broth has been attributed to the influence of these phenolic pigments (Elias et al., 1979; Mondragon and Gonzales, 1978).

Litte is known about the influence of the protein itself. Seidl et al. (1969) isolated a globulin from kidney beans which was resistant even after heating to digestion by 10 different proteolytic enzymes, and Romero and Ryan (1978) also observed low digestibility of an isolated G_1 globulin when compared to that of denaturated bovine albumin. The beneficial effect of denaturation was also recently observed by Liener and Thompson (1980), who studied the digestibility of the G_1 fraction both in vitro and with rats. Evans et al. (1974) and Sgarbieri et al. (1979) observed reduced availability of the sulfur amino acids in rats fed autoclaved beans. Evans and Bauer (1978) also indicated the existence of a dialyzable toxic compound in the soluble fraction of the cooking broth.

This paper reports research on the composition and digestibility behavior of different bean protein fractions and the effect of heat on them. Results indicating the existence of a heat-stable trypsin inhibitor are also reported.

EXPERIMENTAL SECTION

Materials. Beans (*P. vulgaris*) of different varieties were obtained from the Agronomy School of Lavras. Bovine trypsin twice crystallized (Type III; 10 000 BAEE units/mg), pepsin of hog stomach (twice crystallized; 2500 units/mg), pancreatin from hog pancreas (Grade VI), and Pronase (Type VI) were purchased from Sigma Chemical Co. All other compounds were reagent grade. Deionized

Departmento de Alimentos e Nutricão Experimental, Faculdade de Ciências Farmacêuticas, Universidade de Sao Paulo, Conj. das Químicas, B. 14, São Paulo, SP, Brazil.

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