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A Research Note

Tannin in Common Beans: Methods of Analysis and Effects on Protein Quality

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ABSTRACT

Condensed tannins and related phenols in 13 samples of red Guatamalan common beans (*Phaseolus vulgaris*) were determined by four chemical assays. The results were highly correlated although the degree of variation among the samples differed greatly according to the assay. In rat feeding trials, tannin content was negatively correlated with net protein ratio, a measure of protein quality, and positively correlated with protein digestibility. Neither correlation was statistically significant due primarily to the low tannin content of the diet. Methionine supplementation not only improves the protein quality but may also play a role in metabolic detoxification of tannin.

INTRODUCTION

COMMON BEANS (*Phaseolus vulgaris*) and other food legumes contain variable amounts of polyphenols such as condensed tannins (Ronnenkamp, 1977; Bressani and Elías, 1980; Price et al., 1980). Their effect on the nutritional value of food legumes is not well established. They may decrease protein quality by decreasing digestibility (Elías et al., 1979; Bressani and Elías, 1980; Marquez et al., 1981). Cooking, besides destroying trypsin inhibitors and hemagglutinins, also partially removes polyphenols, which results in increased protein quality (Elías et al., 1976). Polyphenols may provide agronomic benefits such as protection of the seed upon germination (Ma and Bliss, 1978). These preliminary findings warrant further studies on polyphenolic compounds in beans. Techniques which measure different characteristics of polyphenols are now available and are of interest for preliminary characterization of polyphenols from food legumes. We have, therefore, applied a variety of assays for polyphenol content to Guatemalan red beans, and correlated the results with protein quality evaluation performed on the same materials.

MATERIALS & METHODS

RED COMMON BEANS (*Phaseolus vulgaris*) were collected from rural markets in Guatemala. A 5-kg sample was stored in the laboratory at 4°C.

The uncooked samples were analyzed by the method of Burns (1963) using the Folin-Denis reagent; this assay measures total phenols expressed as weight equivalents of tannic acid which was used to standardize the assay. Another polyphenol assay was the vanillin-hydrochloric acid method of Burns (1971), referred to as the uncorrected vanillin assay; it was standardized with catechin, so the results are expressed as catechin equivalents. Samples were also analyzed by the vanillin assay as modified by Price et al. (1978), using a blank to eliminate interference by seed pigments, and for protein precipitable phenols as described by Hagerman and Butler (1978). Purified sorghum tannin (Hagerman and Butler, 1980) dissolved in methanol was used to prepare standard curves in these assays, from which % tannin was calculated.

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About 4 kg of each sample were cooked in 12L of water for 30 min under 15 lb pressure at 121°C and, after drying, the beans were ground to a flour through a 40 mesh screen with a hammer mill. The cooked beans were analyzed for nitrogen (AOAC, 1970) and for methionine by microbiological assays using *Leuconostoc mesenteroids* (Steel et al., 1949). Diets contained bean flour equivalent to 10% protein, 4% mineral mixture (Hegsted et al., 1941), 1% cod liver oil, 5% refined cottonseed oil and corn starch to adjust to 100%. All diets including a casein control and a protein-free diet were supplemented with a complete B-vitamin mixture (Manna and Hauge, 1953).

The diets and water were fed ad libitum to groups of eight weanling rats in two trials of 4 animals each, housed in individual all-wire screen cages. The protein assay procedure used was the net protein ratio (NPR) (Bender and Doell, 1957). The experimental period was 14 days, measuring weight changes and food consumed every 7 days. For an additional 5 days, feed intake was kept as constant as possible for digestibility assays, in which all fecal matter was collected, dried, weighed and analyzed for nitrogen. Apparent protein digestibility was calculated by subtracting the nitrogen excreted in feces from the nitrogen ingested. The results were analyzed using standard statistical methods (Snedecor, 1946).

RESULTS

THE PROTEIN, methionine, and polyphenol content of the samples is shown in Table 1. A sixfold difference in the apparent tannin content is observed using the uncorrected vanillin assay, which detects condensed tannin and monomeric flavan-3-ols such as catechin, and other extractable phenols and pigments. In contrast, the Folin-Denis assay for total phenols showed relatively little variation among samples. The marked difference in variability using these two assays illustrates the importance of selecting appropriate chemical techniques for estimating biological effects of polyphenols. Three- to fourfold differences in apparent tannin content were obtained with the other, more specific, assays. Polyphenolic compounds as determined by the uncorrected vanillin assay are highly correlated (0.1% level) with tannins determined as protein precipitable phenols ($r = 0.920$) and the modified vanillin assay ($r = 0.882$) and at the 1% level with total phenols ($r = 0.780$). Tannin determined as protein precipitable phenols is highly correlated (0.1%) to tannin as determined by the modified vanillin assay ($r = 0.810$). The correlation between total phenols and tannin by the modified vanillin ($r = 0.549$) and the precipitation methods ($r = 0.661$) is significant only at the 5% level.

The protein quality of the beans with and without methionine addition is shown in Table 2. Without methionine, the NPR varied from 1.42–2.01, compared to 3.98 for casein. There was a highly significant increase in NPR upon methionine supplementation, but the increase was not equal for all bean samples.

DISCUSSION

THE NUTRITIONAL SIGNIFICANCE of polyphenols in common beans can not be clearly established without adequate methods for their assay. Polyphenol levels in common beans are quite variable and are related to the color of the seed, with white containing very low amounts, while red, black and bronze, have significantly higher levels

Table 1—Protein, methionine, and polyphenol content of red beans

Sample no.	Protein (% N x 6.25)	Methionine g/g N	Protein precipitable phenols (% Tannin)	Modified vanillin assay (% Tannin)	Folin-Denis assay (Tannic acid equivalents) (g/100g)	Uncorrected vanillin assay (Catechin equivalents) (g/100g)
1	25.4	0.062	0.44	0.68	0.91	1.59
2	20.9	0.062	0.25	0.57	0.77	0.66
3	23.8	0.060	0.26	0.60	0.86	0.98
4	22.2	0.068	0.35	0.84	0.77	1.30
5	23.5	0.066	0.52	1.12	0.91	2.54
6	22.9	0.069	0.16	0.32	0.70	0.40
7	20.4	0.058	0.20	0.37	0.87	0.56
8	23.4	0.067	0.38	1.04	0.95	2.10
9	21.2	0.062	0.37	0.73	0.92	1.48
10	18.4	0.079	0.28	0.59	0.89	1.10
11	19.8	0.064	0.30	0.60	0.81	1.05
12	22.0	0.063	0.30	0.79	0.93	1.62
13	24.5	0.062	0.38	0.56	0.95	1.78
Mean ± Std. Dev.	22.2 ± 4.0	0.065 ± 0.005	0.32 ± 0.10	0.68 ± 0.23	0.86 ± 0.08	1.23 ± 0.62
Range	18.4 – 25.4	0.058 – 0.079	0.16 – 0.52	0.32 – 1.12	0.70 – 0.95	0.04 – 2.45
C.V. %	18.0%	8.2%	30.7%	33.9%	9.2%	46.9%

Table 2—Weight gain, apparent protein digestibility and NPR of red beans

	Avg wt gain (g)	NPR	Apparent protein digest. (%)
	No methionine added		
Mean ± Std. Dev.	6.9 ± 1.5	1.72 ± 0.16	72.9 ± 1.8
Range	4 – 9	1.42 – 2.01	70.0 – 76.0
C.V. %	21.7%	6.3%	2.4%
Casein	38 ± 5	3.98 ± 0.31	93.4 ± 0.6
	0.3% Methionine added		
			NPR increase, %
Mean ± Std. Dev	30.7 ± 3.6	3.46 ± 0.21	203 ± 27
Range	24 – 36	3.16 – 3.85	166 – 262
C.V. %	11.6%	6.0%	13.1%

(Bressani and Elías, 1980). Thus only red-colored beans were used in these studies.

The four assays differed in their capacity to detect various types of polyphenols, although the values were highly correlated and fall within the range of values reported by others using similar methods.

Because legume tannins bind dietary protein (Ariga et al., 1981) and inhibit proteolytic activity in the gastrointestinal tract (Griffith and Moseley, 1980), a negative correlation between protein digestibility and tannin content was expected, rather than the positive but statistically nonsignificant correlation (r = 0.01 to 0.45) observed. Negative correlations between tannin content and protein digestibility in legumes have been reported by some, but not all, previous workers (Bressani and Elías, 1980), while negative correlations are always found in sorghum (Cummings and Axtell, 1973). The absence of an effect on protein digestibility may have been due to low polyphenol levels in the diets. Their content in beans (Table 1) is relatively low compared to the amount found in high-tannin sorghums, which may contain 2% tannin as assayed by the modified vanillin assay. Furthermore, the polyphenol content was further diluted when starch was added to beans to reduce protein content to 10% for feeding studies. A further decrease in polyphenol content occurs due to the cooking process used to destroy antiphysiological substances in beans. Apparent losses of 20–39% from raw to cooked when expressed as tannic acid and of 61–98% when expressed as catechin equivalent have been reported (Bressani et al., 1982).

The results from the four tannin assay methods were negatively correlated with net protein ratio, confirming reports by other workers (Ronnenkamp, 1977; Bressani and Elías, 1980) but the correlations were not statistically significant (r = −0.08 to −0.29). Polyphenol content was positively correlated with the NPR values of the methionine supplemented bean diets but the correlation was not statistically significant (r = 0.10 to 0.39). Beans with a higher polyphenol content generally responded more to methionine addition than those with lower contents. The regression of uncorrected vanillin to percentage increase in quality on adding methionine gave a positive correlation (r = 0.364) which was statistically not significant. Since the methionine content varied little among samples (Table 1) the differential effect of methionine was probably due to other reasons. These results suggest that small amounts of polyphenolic compounds may be absorbed and detoxified in a process which utilized methionine as a methyl donor and which increases the methionine deficiency of bean protein. According to this hypothesis, to be tested by future research, methionine addition should increase protein quality more for high-tannin beans than for low-tannin beans, as we observed. Thus, added methionine is postulated not only to improve the quality of the bean protein but also to aid in tannin detoxification.

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