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POLYPHENOLS IN CEREALS AND LEGUMES

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The Nutritional Role of Polyphenols in Beans

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The nutritional role of polyphenols in food legumes remains unclear. White, black, and red *Phaseolus vulgaris* contain 0.34–0.42, 0.57–1.15, and 0.95–1.29% polyphenols as tannic acid mainly in testa; protein quality is higher for white, black, and red, respectively. Cooking destroys known antiphysiological factors, but not tannins, which are partially removed with cooking liquor. Bean protein quality is lower when assayed with cooking liquor, for red and black, but not white. Polyphenols decrease protein digestibility in animals and humans, probably by making protein partially unavailable or by inhibiting digestive enzymes and increasing fecal nitrogen.

Legume foods provide the supplementary protein to diets based on either cereal grains or starchy food, commonly consumed in developing countries. Thus, increasing attention is being given to agricultural programs aimed at increasing yield and, hopefully, availability to consumers.

It is important, however, to consider in such programs the introduction of improved nutritional-quality characteristics as well as acceptable cooking and organoleptic qualities (Hulse et al. 1977).

Much attention has been given in the past to the destruction of the well-known antiphysiological factors in legume foods, such as trypsin inhibitors, by appropriate processing. Likewise some reports have focused on the establishment of nutritional standards (Hulse et al. 1977), which, besides protein and specific essential amino acids, include other characteristics related to acceptability and ease of preparation for consumption. On the other hand, polyphenolic compounds in food legumes have not been as thoroughly investigated, and there are only a few studies in animals which indicate that these compounds affect nutritional quality (Chang and Fuller 1964; Lindgren 1975; Marquardt et al. 1978). However, there is need to know more about their possible role in relation to storage, cooking quality, and nutrient utilization. Legume grain protein has a low digestibility, which has not been adequately explained; furthermore, after long storage legumes become difficult to cook and are, therefore, refused by the consumer. Finally, although mothers in developing countries do not feed whole cooked legume foods to their children because of their undesirable effects, they do often feed cooking broth to 1- to 3-year-old children (Bressani et al. 1973). The question was then raised as to whether polyphenolic compounds in common beans and other legume foods play a role in the constraints indicated above.

Polyphenol Content in *Phaseolus vulgaris* Strains

Polyphenolic compounds in common beans have been determined either by the method of Folin-Denis (Joslyn 1970) and expressed as tannic acid, or by the vanillin-HCl method of Burns (1971) and expressed as catechin equivalents (CE). Reports on the subject are relatively few, however those available show similar results as shown in Table 1. The materials in the table represent strains of common beans grown in Wisconsin, Puerto Rico, and Guatemala. When the polyphenols were determined by the Folin-Denis method, the values were higher than when expressed as catechin equivalents. Independent of how the results were calculated, however, they indicate differences with respect to seed coat colour. White beans show the lowest amounts of polyphenols, which in general increase in black, red, and bronze varieties. The variability in white-coated beans is relatively small, however it is much greater in materials with black, red, and bronze seed coats. Studies on tannin-content inheritance in common beans have shown that it has a high broad-sense heritability (Ma Yu and Bliss 1978; Ronnenkamp 1977), therefore, low-

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Table 1. Polyphenol content in *P. vulgaris* cultivars.

Sample origin	n	Testa colour	Catechin equiv. value (mg/g)	Tannic acid (%)
Wisconsin[1]	6	white	2.31 (1.50-3.26)	—
	4	black	6.65 (2.48-18.86)	—
	3	bronze	7.80 (7.10-8.16)	—
Puerto Rico[1]	1	white	2.40	—
	9	black	5.30 (3.40-10.60)	—
	4	red	12.56 (7.18-15.10)	—
Guatemala[2]	31	white	—	0.38 (0.16-0.53)
	249	black	—	1.13 (0.72-1.77)
	39	red		1.14 (0.87-1.52)
Guatemala[3]	3	white	0.24 (0.18-0.28)	
	10	black	1.99 (0.62-5.90)	
	4	red	6.42 (1.87-10.06)	
	3	bronze	9.19 (0.38-14.10)	

[1]Ma Yu and Bliss 1978.
[2]Bressani and Braham 1978.
[3]Linares and de Bosque 1978.

Table 2. Polyphenols in red-coloured beans expressed as tannic acid and as catechin equivalent.

Cultivar No.	Tannic acid (%)	Catechin equiv. value (mg/g)
1	0.91	15.9
2	0.77	6.6
3	0.86	9.8
4	0.77	13.0
5	0.91	25.4
6	0.70	4.0
7	0.82	5.6
8	0.95	21.0
9	0.92	14.8
10	0.89	11.0
11	0.81	10.5
12	0.93	16.2
13	0.95	17.8
$\bar{X}$	0.87	13.2
r	0.65**	

tannin strains in coloured cultivars may be obtained either by selecting among existing pure lines, or by crossing and selecting for appropriate recombinations. The importance of this finding is that since populations have strong colour preferences, it is possible to select coloured seeds with low polyphenol content, meeting at the same time consumer preferences, such as colour and flavour.

With respect to the analytical methodology for polyphenol compounds in grain legumes, there is a high correlation between "tannic acid" (Folin-Denis) and catechin equivalent (vanillin-HCl) (Bressani et al., in preparation) as shown in Table 2. In this case, 13 red-coloured cultivars were analyzed by both methods. It would be desirable, however, to have a more specific methodology and to know exactly the type of phenolic compounds being analyzed.

Tannins in common beans are located in the seed coat of the grain, with low or negligible amounts in the cotyledons. Some results in this respect are shown in Table 3. The values reported were done on cotyledons without seed coat, on the seed coat, or on the whole seed. The results show that the cotyledons contain lower concentrations than the whole seed, while the seed coat is the main source of polyphenolic compounds in common beans, whether expressed as catechin equivalent or tannic acid. According to this, there should be a correlation between percentage seed coat and polyphenol content, and between polyphenol content and seed size. However, the various studies reported did not show such a relationship, indicating that phenolic content is independent of seed size (Ma Yu and Bliss 1978; Elías et al. 1979).

Effects of Processing

Common beans are cooked before consumption to make them soft and to destroy antiphysiological substances. Though the cooking process may vary, it generally follows the sequence of steps shown in Fig. 1. Soaking beans in water for

Table 3. Polyphenol distribution in anatomical fractions of *P. vulgaris*.

Sample origin	Testa colour	Whole seed	Cotyledons	Seed coat
Catechin equiv. value (mg/g)				
Wisconsin[1]	white	2.31	2.17	—
	black	6.65	2.90	—
	bronze	7.80	2.04	—
Puerto Rico[1]	white	2.40	0.44	—
	black	5.30	0.98	—
	red	12.56	1.01	—
Tannic acid (%)				
Guatemala[2]	white	3.85	4.15	1.30
	black	7.95	5.25	42.50
	red	9.30	5.00	38.00

[1]Ma Yu and Bliss 1978.
[2]Elías et al. 1979.

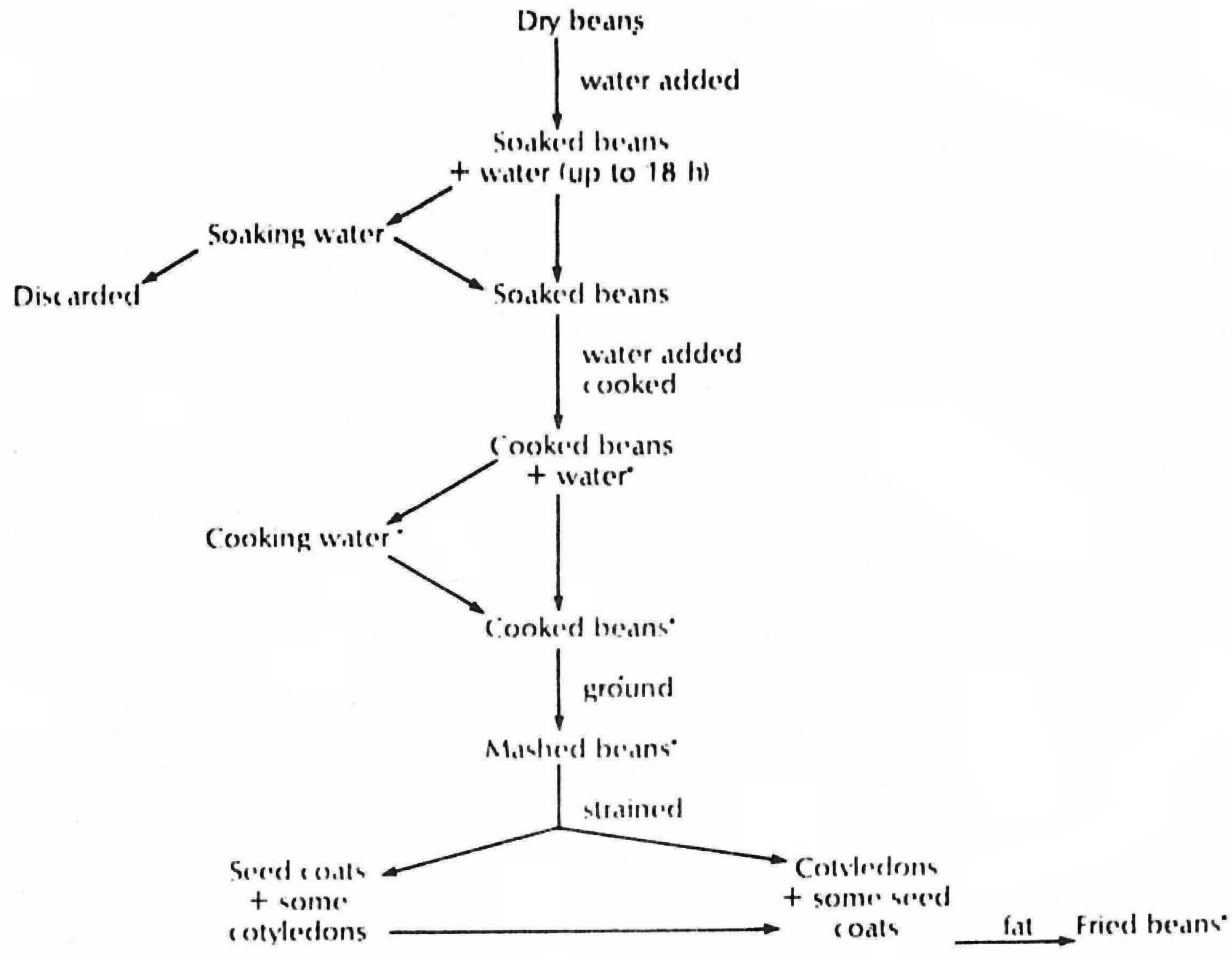

Fig. 1. Processing of beans for consumption in Latin America. (Asterisks indicate fractions and/or products consumed.)

periods up to 18 h is a common practice in Latin America. The soaking water, which contains around 1.3% solids, may or may not be discarded. Cooking is carried out in additional water for periods of up to 4 h at atmospheric pressure, or for about 30 min under 15 pounds (6.8 kg) of pressure. The cooking water may be removed to be used as a soup, particularly for children, or it may be left in with the beans. Cooking water alone usually contains 4–10% solids. Beans may be then crushed and consumed or strained and fried (Bressani et al. 1973).

Several studies have been carried out to learn of the changes which may take place during cooking. Table 4 summarizes results of cooking black, red, and white bean samples. The cooked material was analyzed without removing the cooking water. It can be seen that polyphenols expressed as tannic acid decreased 30 49% on cooking (de España 1977). The degree of change is variable and at present its nature cannot be explained.

Because no physical separation was made, the change may have resulted from the binding of polyphenols with other organic substances, or from alterations in chemical structure of the polyphenols, thus rendering them incapable of giving the chemical colour reaction measured by the Folin-Denis method.

Table 4. Losses of polyphenols during cooking, expressed as tannic acid (de España 1977).

Bean colour	Tannic acid (%) Raw	Cooked	Loss (%)
Black	0.90	0.63	30.0
Red	1.21	0.62	48.8
White	0.40	0.27	32.5

Large amounts of polyphenols may be found in the cooking waters as indicated in Table 5. In these examples, the samples were analyzed raw, and cooked, with and without the cooking broth. The results show lower values in the cooked bean samples analyzed without the cooking broth (Elías et al. 1979; Fukuda Suzuki 1978). The lower section of the table shows the comparatively high amounts found in the cooking broth. Similar studies have been performed, expressing

Table 6. Losses in polyphenols during cooking, expressed as catechin equivalent value, mg/g (Linares and de Bosque 1979).

Bean colour	Raw	Cooked
Black	1.99	0.24
Red	6.42	0.12
White	0.24	0.12
Brown	9.19	0.38

polyphenolics as catechin equivalents, and some results are shown in Table 6. Losses in this case appear to be much higher than when tannic acid is used to indicate polyphenol content (Linares and de Bosque 1979). As indicated, no explanation is available at present to account for the loss. It is possible the polyphenols react with carbohydrate, protein, or other substances which could be measured biologically, or may be partially destroyed.

Attempts to obtain some explanation have been made by following the changes which occur during the processing of the raw beans to crushed cooked beans. The results, as shown in Table 7, indicate that although relatively large amounts of polyphenols could be eliminated by discarding washing and cooking waters, the residue, mainly cotyledons, retains large quantities, because of the apparent migration of the tannins from the seed coats to the cotyledons. The actual amounts ingested will thus depend on how beans are processed and consumed.

Nutritional Role

It is recognized that tannins alter the nutritional quality of plant products. This has been very well demonstrated for bird-resistant grain sorghum (Chang and Fuller 1964; Lindgren 1975).

Table 5. Polyphenols in raw and cooked *P. vulgaris* with and without the cooking broth, expressed as tannic acid.

Colour of seed	Raw	Cooked cooking broth	Cooked + cooking broth	Cooking broth
White[1]	0.35	0.10	0.20	—
Black[1]	0.75	0.34	0.45	—
Red[1]	0.97	0.49	0.45	—
White[2]	0.38	0.20		0.78
Black[2]	0.80	0.52		0.76
Red[2]	0.93	0.41		2.10

[1]Fukuda Suzuki 1978.
[2]Elías et al. 1979.

Table 7. Distribution of polyphenols as tannic acid during cooking of *P. vulgaris*[1], grams.

Bean product	Bean colour		
	Black	White	Red
1) Raw beans	4.50	1.80	7.35
2) Soaking water	0.16	0.05	0.25
3) Soaked beans	3.80	1.94	5.23
4) Soaking water + soaked beans	4.43 (3.96)[2]	2.11 (1.99)	5.78 (5.48)
5) Cooked beans	2.72	1.20	2.75
6) Cooking water	0.86	0.28	0.86
7) Cooked beans + cooking water	3.37 (3.58)[2]	1.48 (1.48)[2]	4.19 (3.61)[2]
8) Mashed beans	3.58	1.48	4.67
9) Strained	(0.73%)[3]	(0.34%)[3]	(0.74%)[3]
10) Seed coat	(1.00%)	(0.28%)	(1.27%)
Loss (1–8) (%)	20.4	17.8	36.5

[1]Values represent absolute amounts from an initial sample of 500 g of beans.
[2]Values in parentheses represent calculated result.
[3]Content in protein.

Table 8. Protein digestibility of beans of different colour.

Bean colour	Tannin (%)[1]	Dig. (%)[1]	Tannin (%)[2]	Dig. (%)[2]
Black	0.63	70.7	0.34	74.6
Red	0.62	68.5	0.49	70.1
White	0.27	75.2	0.10	82.5

[1]de España 1977
[2]Fukuda Suzuki 1978

Table 9. Correlation coefficients between polyphenols as catechin equivalent and in vivo protein digestibility (Linares and de Bosque 1979).

All samples	n = 80	r = 0.3955**
Black	n = 40	r = 0.369
Red	n = 12	r = 0.1031
Bronze	n = 16	r = 0.3987
White	n = 12	r = 0.1264

Some reports have indicated that tannins in different cultivars of peas, field beans, and other food legumes were responsible for lower digestion coefficients for crude protein in poultry (Lindgren 1975; Marquardt et al. 1978).

Artificial rumen studies have shown that tannin extracts from carobs inhibit cellulolytic and proteolytic activity (Tamir and Alumot 1969). Furthermore, the carob tannins have been shown to be strongly inhibitory of trypsin and amylase and, to a lesser degree, of lipase. These results suggest that the depressing effect of tannins results from their action on digestive enzymes. However, they can react with food proteins, interfering with their digestion by enzymes, and, consequently, lowering amino acid availability (Haslam 1974).

Some information on the nutritional role of polyphenols in *Phaseolus vulgaris* has already been obtained. Table 8 describes results from two independent studies showing a relation between polyphenols as tannic acid and protein digestibility (de España 1977; Fukuda Suzuki 1978). In both studies white-coated cultivars showed the highest digestibility and the lowest tannic acid content. Furthermore, red-coloured beans in both studies showed the lowest protein digestibility with the highest tannic acid content. It should be indicated that in both studies the cooking broth was separated from the cooked cotyledons. Larger numbers of samples have been studied recently and Table 9 presents the correlations found between phenolic content and in vivo protein digestibility. These are significantly negative for all samples taken together as well as for black-coated beans. All other correlations are negative although not statistically significant (Linares and de Bosque 1979). As shown previously, the broth contains relatively large amounts of polyphenols and, if included with the cotyledons, it may decrease digestibility further (see Table 10). The addition of the cooking broth to both black- and red-coloured beans decreased

Table 10. Effect of cooking broth addition on protein digestibility of beans of different colour (Elías et al. 1979).

Sample	Protein digestibility (%)		
	White	Red	Black
Cooked beans	81.3 ± 1.8	78.7 ± 2.8	77.9 ± 2.5
Cooked beans + broth	81.4 ± 1.5	70.4 ± 4.8	75.0 ± 3.5

Table 11. Effect of cooking broth addition on average weight gain and protein efficiency ratio (PER) of various food legumes (Elías et al. 1979).

Identification	Ave. wt. gain		PER	
	+ cooking broth	cooking broth	+ cooking broth	cooking broth
Sensuntepeque (black) *(P. vulgaris)*	6	29	0.20	0.88
S-184-N (black) *(P. vulgaris)*	1	29	—	0.95
Red-70 (red) *(P. vulgaris)*	8	10	0.31	0.48
27-R (red) *(P. vulgaris)*	21	27	0.61	0.86
Cowpea *(V. sinensis)*	32	52	1.04	1.24
Soybean *(G. max)*	94	113	1.69	1.80
Pigeon pea *(C. cajan)*	36	51	0.98	1.46

protein digestibility, however such an effect was not obtained with white-coloured beans. It should be indicated, however, that differences in digestibility may not be due to tannins alone. Availability of amino acids may also play a role still to be demonstrated.

The effect of adding the cooking broth is also seen in terms of protein quality as determined by the protein efficiency ratio (PER). Some representative values are shown in Table 11. In this case, the elimination of the broth as part of the bean in the diet resulted in higher PER values. This is clear for *P. vulgaris*, as well as for other food legumes, such as cowpeas, soybeans, and pigeon peas, although not as striking as for *P. vulgaris* (Elías et al. 1976). It is of importance to recognize that the effect may not be entirely due to tannins but may result from interactions of the protein with some unidentified adverse component of the broth.

The results of two studies of interest are shown in Table 12 (de España 1977; Fukuda Suzuki 1978). In the first, there is a direct relationship between polyphenol content and the net protein ratio (NPR) value of the legume protein. The relationship between tannin content and protein quality is also evident when the protein is supplemented with methionine, thus eliminating the possibility of the role this amino acid plays in the protein quality of legume foods. In the second study, the assay utilized was the nitrogen growth index (or NGI, which is determined as the slope of the line relating growth rate to protein intake). The assay is carried out by feeding rats diets with different levels of protein from the same source. As more beans form part of the diet, more phenolics are included in it. The results obtained suggest again that coloured beans have a lower protein-quality value than white beans. Additional studies are now under way to ascertain the relationship between polyphenols in beans and protein digestibility.

Studies have also been conducted with adult human subjects (Hernández 1979). Preliminary results from four subjects show that black beans had the lowest protein digestibility, followed by red beans, while both were lower than the digestibility value of cheese protein (see Table 13). These studies are now being expanded to include white beans with and without the cooking broth from

Table 12. Relationship between polyphenol content in beans and their protein quality with and without the addition of methionine to the diet.[1]

	Tannin[2] (%)	methionine	+ methionine
Net protein ratio (NPR)			
Black[3] $n = 5$	0.47 0.81 (0.63)	2.00	3.18
Red[3] $n = 2$	0.59 0.64 (0.62)	1.96	2.88
White[3] $n = 2$	0.27 0.28 (0.27)	2.38	3.90
Nitrogen growth index (NGI)			
Black[4] $n = 3$	0.29 0.38 (0.34)	1.81	—
Red[4] $n = 2$	0.48 0.50 (0.49)	2.09	—
White[4] $n = 2$	0.08 0.11 (0.10)	2.56	--

[1]Both studies conducted without adding the cooking broth.
[2]In cooked sample.
[3]de España 1977.
[4]Fukuda Suzuki 1978.

Table 13. Protein digestibility in humans fed bean protein with different content of polyphenols, expressed as tannic acid (Hernández 1979).

Protein source	Nitrogen balance (mg/kg/day)	Digestibility: Apparent (%)	Digestibility: True (%)
Red beans	46.6 ± 6.5	55.7 ± 4.7	78.9 ± 4.4
Black beans	46.4 ± 2.8	49.6 ± 2.4	72.8 ± 3.1
Cheese	72.0 ± 3.4	76.2 ± 1.4	98.3 ± 1.6

either red or black beans. In order to find out whether or not tannins are influencing the results, regression equations were calculated, between catechin intake and fecal nitrogen. Fig. 2 shows the results of two regressions. The first, calculated with fecal N excretions from a diet without beans or tannins, and the second, in which this point was not included. There is a statistically significant correlation in the first case. However, in the second case the correlation is not statistically significant. It is believed that there is need to increase the number of observations to be able to be conclusive on this particular point.

In summary, it may be concluded that tannins affect the utilization of protein in beans through increasing fecal N output, as made evident in rat studies. Trials with human subjects showed a similar trend, although the effect was less marked than in rats.

Based on the observations reported in this paper and on those from the literature, it may be suggested that the polyphenol compounds in common beans decrease protein digestibility either by inhibiting digestive enzymes or by reacting with protein, reducing amino acid availability, or both. The decrease in protein digestibility, in turn, reduces absorption of amino acids which could explain the lower protein-quality value observed. In case phenolic compounds are absorbed, they will have to be eliminated, either as glucuronate derivatives or sulfates; in the latter case, sulfur amino acid needs will increase, thus decreasing the quality of the protein for growth purposes. All these effects are, however, relatively small.

With respect to the significance of the polyphenols present in beans and their effect in nutrient utilization, the problem should be analyzed from two points of view. One is the nutritional effect per se and the second, bean acceptability. As for the nutritional effects, these are evident, but relatively small. They would probably be smaller considering that beans are only one component of the diet, thus the effects will be diluted or minimized, and certainly, very difficult to measure.

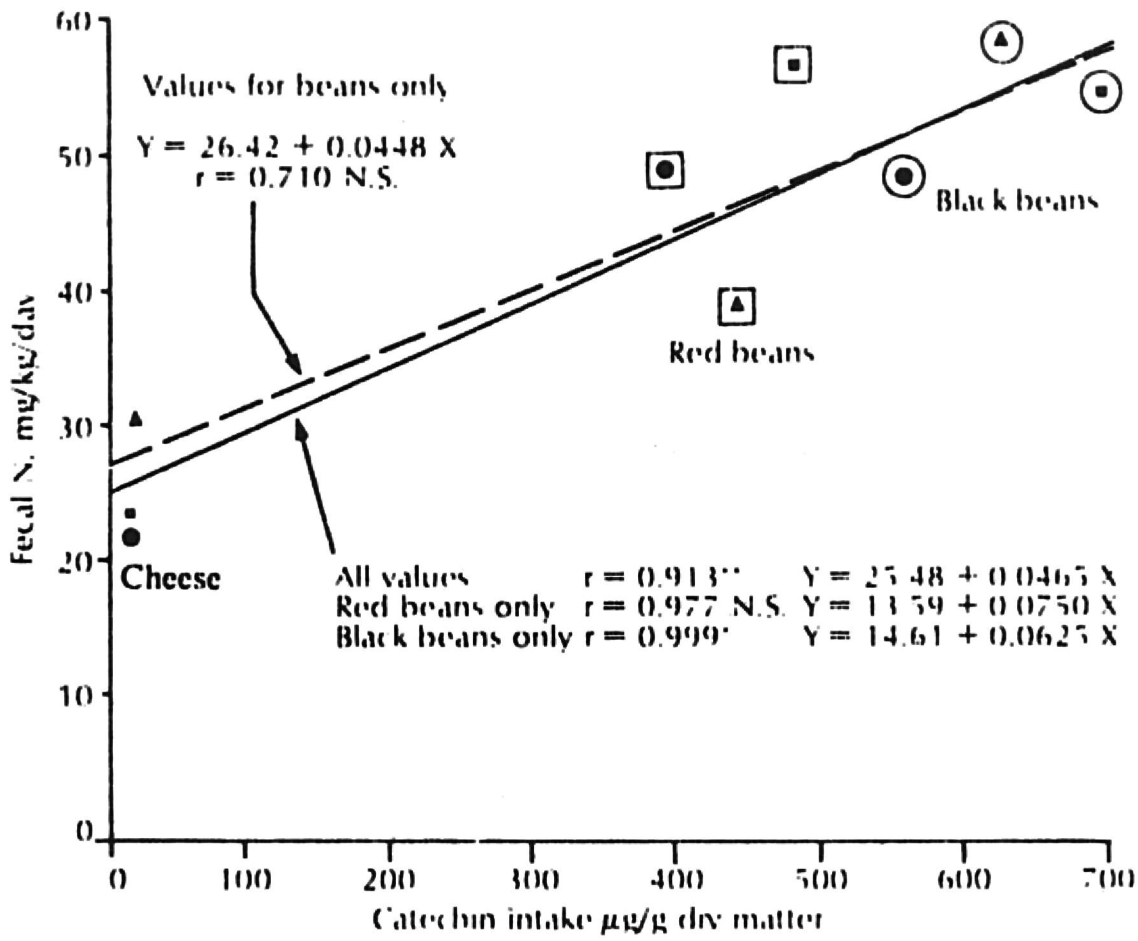

Fig. 2. Relationship between catechin intake and fecal N output in humans (Hernández 1979).

Regarding the problem of bean acceptability, one aspect is clear, at least in Latin America, coloured beans are greatly preferred to white-coated beans, by all populations. Furthermore, the cooking liquors must be thick and dark and are preferred for young children. The question could be asked if acceptability is based only on colour of the seed coat or also on the small amounts of polyphenolic compounds present. It is of interest to point out in this respect that preference varies even within the same colour. Therefore, it would be of interest to find out if phenolic compounds in common beans are related to acceptability and to increase our knowledge of their nutritional role, before genetic selection is undertaken by legume breeders to eliminate them.