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Received for review January 3, 1977. Accepted April 12, 1977.

Relationship between Tannin Levels and in Vitro Protein Digestibility in Finger Millet (*Eleusine coracana* Gaertn.)

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Determination of the total phenol and tannin levels of finger millet varieties indicated wide variations in phenolic contents. White-grain varieties had lower phenolic content than the brown-grain varieties. In vitro protein digestibility values of low tannin samples were higher than those of the high tannin samples. Dehulling had the effect of removing most of the phenolics from finger millet grain with concomitant increase in in vitro protein digestibility. Addition of tannic acid to low tannin or dehulled finger millet samples decreased the in vitro protein digestibility. Tannins were found to be associated mostly with the glutelin fraction of finger millet protein.

Utilization of protein in animal and human diet is adversely affected by phenolic constituents and tannins (polyphenols) since they have the ability to bind with and precipitate proteins. Growth retardation has been observed in chicks and rats fed on diets containing high tannin sorghum (Chang and Fuller, 1964; Fuller et al., 1966; Jambunathan and Mertz, 1973). Tannins have been shown to be growth depressing and toxic to chicks (Vohra et al., 1966). Availability of amino acids in high tannin sorghums is reported to be much lower than in low tannin sorghums (Rostango, 1972). Extraction of tannins from high tannin sorghums results in increased weight gain and feed efficiency of chicks and rats (Armstrong et al., 1974; Featherstone and Rogler, 1975). Addition of phenolics to diets decrease the nutritive value of proteins (Horigome ad Kandatsu, 1968). Further, tannins of sorghum cause a reduction of in vivo and in vitro dry matter disappearance and protein digestibility (Stallcup and Davis, 1962; McGinty, 1968; Maxson et al., 1973; Schaeffert et al., 1974; Featherstone and Rogler, 1975).

The occurrence of tannins in cereals is rare and has so far been reported only in strains of barley and sorghum. Tiemann and Blumenberg (1959) reported that among some cereals tested tannin-like substances were present only in barley while these were absent from wheat, rye, oats, and maize. They reported, however, that all these cereals contained chlorogenic acid.

Finger millet (*Eleusine coracana* Gaertn.) is an important food crop of India and Africa. The protein quality of finger millet varieties has been the subject of earlier studies from this laboratory (Virupaksha et al., 1975). This report deals with the tannin and phenolic contents and their relation to the in vitro protein digestibility (IVPD) in finger millet.

MATERIALS AND METHODS

Sample. Thirty-two samples of finger millet consisting of 19 Indian, 10 African, and 3 Indian × African cross-bred varieties were procured from the germ plasm collection of the Millet Research Centre, University of Agricultural Sciences, Hebbal, Bangalore, India. Samples had a range of seed color from white to very dark brown. Samples designated by the prefix HP or HX represent crosses between white-grain and brown-grain varieties.

Samples were ground to a fine powder with a mortar and pestle. Dehulling of the finger millet varieties was performed by soaking the grain for 1 min in concentrated H_2SO_4 and pouring tap water on the acid. This treatment loosened the pericarp which was rubbed off the grain by hand and removed by decantation with large volumes of water. The dehulled grain was air-dried at room tem-

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Table I. Content of Total Phenols and Tannins[a] of Finger Millet Varieties

Variety	Seed color	Total phenols (Folin-Denis), (%)	Tannins (Catechin equivalent (%)
Indian			
Hamsa	W	0.08	0.06
HPW 27-4	W	0.09	0.04
HPW 83-4	W	0.07	0.04
ECW 854	W	0.06	0.06
ECW 955	W	0.09	0.05
HX 799	W	0.09	0.04
Purna	B	0.74	0.85
HPB 20-5	B	0.53	0.57
HPB 1-8	B	0.71	1.03
HPB 7-6	B	0.62	0.84
HPB 23-6	B	0.57	0.76
EC 4840	B	0.63	0.92
IE 246	DB	0.37	0.14
IE 328	DB	0.42	0.26
IE 736	DB	0.37	0.12
IE 497	DB	0.55	0.36
IE 860	DB	0.96	1.05
IE 121	B	0.69	0.57
IE 395	B	0.64	0.52
	Mean	0.44	0.44
	SD	0.08	0.14
African			
IE 927	B	2.44	3.47
IE 929	B	2.38	3.42
IE 974	B	0.92	0.85
IE 976	B	0.66	0.76
IE 978	B	0.54	0.50
IE 979	B	1.00	1.14
IE 1029	B	0.85	0.93
IE 1038	B	0.58	0.46
IE 1039	B	0.86	1.04
IE 1065	B	0.61	0.52
	Mean	1.08	1.31
	SD	0.51	1.32
Indian × African			
14-1A-3-26	B	1.08	1.46
91-4-7-18	B	1.48	2.02
HX 929-43-7-3	W	0.10	0.03
	Mean	0.89	1.17
	SD	0.50	1.05

[a] All values on dry weight basis; W = white, B = brown, DB = dark brown.

perature and ground to a fine powder. Moisture was determined in the samples by the standard (AOAC) procedure.

D-Catechin and vanillin were purchased from Sigma Chemical Co., chlorogenic acid was obtained from Koch-Light Laboratories Ltd., United Kingdom. Tannic acid was procured from W.G. Bush and Co., London. Pepsin (1:3000, B.P.C. 1959) was purchased from G.M. Laboratories, India.

Estimation of Total Phenols and Tannins. One to two grams of finger millet flour or dehulled finger millet flour were extracted with 50 mL of 1% HCl in methanol for 24 h at room temperature with occasional swirling. Total phenols were estimated in aliquots of the extract using the Folin-Denis reagent by the procedure of Swain and Hillis (1959). Chlorogenic acid was used as the reference standard for the estimation of total phenols. Polyphenols (tannins) were estimated in the extract by the vanillin–HCl procedure according to Burns (1971), using D-catechin as the standard. Total phenol and tannin contents were expessed as grams per 100 g dry flour (Table I). Statistical analyses were conducted according to the methods described by Snedecor and Cochran (1967).

Table II. Analysis of Variance

Source of variation	df	Sum of squares	Mean sum of squares
Origin[a]	2	5.51	2.755[b]
Error	29	16.56	0.5710
Total	31	22.07	

[a] Finger millet varieties grouped according to origin as Indian, African, and Indian × African. [b] Significant at the 5% level.

Table III. In Vitro Protein Digestibility of Finger Millet Varieties

Variety	Tannins, %	IVPD, %
Hamsa	0.06	85.1
IE 246	0.14	83.0
IE 121	0.57	88.1
HPB 20-5	0.57	86.5
Purna	0.85	78.0
EC 4840	0.92	86.3
HPB 1-8	1.03	81.3
IE 860	1.05	84.2
IE 979	1.14	85.5
14-1A-3-26	1.46	83.8
91-4-7-18	2.02	84.3
IE 929	3.42	57.4
IE 927	3.47	55.4

Table IV. Effect of Added Tannic Acid on in Vitro Protein Digestibility of the Finger Millet Variety, IE 246

Tannic acid added, %	IVPD, %
0.00	83.0[a]
0.75	78.4
1.50	70.1
2.25	65.8
2.50	62.2
3.00	60.4
3.75	53.2
4.50	48.7
5.00	43.3

[a] Endogenous tannin level of IE 246 is 0.14% (Table III).

In Vitro Protein Digestibility. In vitro protein digestibility was determined by calculating the difference between the amount of nitrogen in the sample before and after hydrolysis with pepsin (AOAC, 1965). Two hundred milligrams of whole seed or dehulled finger millet flour was incubated with 50 mL of 0.2% pepsin in 0.075 N HCl for 24 h at 37 °C. Digestion was performed in duplicate. The digests were filtered through Whatman No. 2 filter paper, and the residue was washed with warm water on the filter. Nitrogen in the residue was estimated by the micro-Kjeldahl method. IVPD was obtained by calculating the difference between the amount of total nitrogen in the sample before and after in vitro digestion with pepsin. Kjeldahl nitrogen was multiplied by the factor 6.25 to obtain crude protein.

Tannic acid was added in graded amounts (0.75 to 5%) to the whole seed or dehulled finger millet flour, and the effect of the added tannic acid on the IVPD was determined as described above.

Association of Tannins with Finger Millet Protein Fractions. The proteins of five varieties of finger millet were separated into three fractions by a modification of the procedure of Landry and Moureaux (1970). Albumin-globulin (fraction A) was extracted from the nondefatted wholeseed flour with 0.5 M NaCl, followed by distilled water. The residual flour was then successively extracted with (i) 70% isopropyl alcohol with 0.6% 2-mercaptoethanol to obtain the prolamin fraction (fraction

Table V. Effect of Dehulling on Total Phenols, Tannins, and in Vitro Protein Digestibility of Finger Millet[a]

Variety	Total phenols, %		Loss of total phenols on dehulling, %	Tannins, %		Loss of tannins on dehulling, %	IVPD, %		Increase in IVPD on dehulling, %
	WS	DH		WS	DH		WS	DH	
Hamsa	0.08	0.03	62.5	0.06	Tr	ca. 100	85.1	91.0	6.9
Purna	0.74	0.16	78.3	0.85	0.09	89.4	78.0	92.3	18.3
IE 929	2.38	0.56	76.5	3.42	0.41	88.1	57.4	93.4	62.7
IE 927	2.44	0.50	79.5	3.47	0.40	88.6	55.4	93.7	69.1

[a] WS = whole seed, DH = dehulled.

Table VI. Effect of Added Tannic Acid on in Vitro Protein Digestibility of Dehulled Finger Millet

Variety	Tannins in dehulled flour, %	IVPD, %	Tannic acid added, %	IVPD, %
Purna	0.09	92.3	0.75	81.2
IE 927	0.40	93.7	3.00	69.2
IE 929	0.41	93.4	3.00	67.2

B) and (ii) borate buffer (pH 10.0) containing 0.5 M NaCl, 0.6% 2-mercaptoethanol, and 0.5% sodium dodecyl sulfate to obtain the glutelin fraction (fraction C) (Virupaksha et al., 1975). Nitrogen in the extracts was determined by the micro-Kjeldahl method and tannin was estimated by the vanillin–HCl method.

RESULTS AND DISCUSSION

Preliminary investigations from this laboratory indicate that catechin and condensed tannins with ability to precipitate gelatin are the main phenolic constituents of finger millet (unpublished results). Tannins in this paper refer to polyphenols capable of binding to protein (Swain and Bate-Smith, 1962) and are estimated by reaction with the vanillin–HCl reagent.

Table I gives the total phenol (Folin-Denis reagent) and tannin (catechin equivalents) levels of 32 varieties of finger millet. The seed coat color of the samples ranged from white to very dark brown. It is seen that the white grain varieties have very low phenol and tannin levels compared with the brown and dark brown varieties (mean tannin content: white, 0.05%; brown, 0.61%). The differences in tannin levels between the white and brown varieties was highly significant ($p < 0.001$) by the Student's t test. Highest amounts of tannins (3.42 and 3.47%) were found in two African varieties, IE 929 and IE 927.

Analysis of variance between varieties grouped according to origin is shown in Table II. The analysis revealed that group differences were significant ($p < 0.05$). However, the differences were observed mainly between the Indian and African varieties rather than between either of them and their hybrids (Indian × African).

IVPD of a few finger millet varieties with tannin levels ranging from 0.06 to 3.47% are shown in Table III. The low tannin varieties showed high IVPD values of about 85%, whereas the high tannin varieties gave low values of IVPD. However, a few varieties with intermediate tannin levels (1.46 to 2.02%) had IVPD values comparable with those of the low tannin varieties. It is possible that the full effect of tannins on protein digestibility is expressed only when the tannin content exceeds a certain threshold value (Fuller et al., 1966).

Results of the effect of adding graded amounts of tannic acid to a low tannin finger millet variety (IE 246) are summarized in Table IV. It is seen that the added tannic acid does have the effect of decreasing IVPD of the finger millet sample. However, it is recognized that tannic acid, a hydrolyzable tannin, may be structurally unrelated to the condensed tannins in finger millet. Since tannic acid binds to proteins, it was of interest to study the effect of added tannic acid on the IVPD of low tannin finger millet varieties.

Figure 1 shows the regression lines of IVPD on tannin levels and IVPD on tannic acid added to finger millet. The analyses show that IVPD is negatively correlated with tannin levels ($r = -0.868$, $p < 0.001$) as well as tannic acid levels ($r = -0.996$, $p < 0.001$) when added to finger millet. The regression lines indicate a decrease in IVPD of 8.58% for 1% increase in tannins and a decrease of 7.89% in IVPD for every 1% increase in tannic acid.

In sorghum most of the polyphenols are shown to be present in the pericarp (testa) layer. Therefore, it was of interest to study the effect of dehulling the finger millet samples on the tannin levels and IVPD. The results of such studies are shown in Table V. It is seen that dehulling had the effect of removing nearly 90% of the polyphenols and 80% of the total phenols from the high tannin finger millet varieties. The IVPD of the high tannin varieties increased concurrently with dehulling and consequent loss of polyphenols from finger millet samples. The gain in IVPD on dehulling was as much as 70% of that of the whole seed.

The effect on IVPD of adding tannic acid to a few dehulled samples of finger millet to approximately the levels of tannins found in the whole seed samples was next investigated, and the results are shown in Table VI. It is seen that the addition of tannic acid to dehulled flour does lower the IVPD, though not to the extent found in the whole seed samples. This difference could be due to the loss on dehulling of other polyphenolic compounds, probably pigments, present in the seed coat.

Table VII. Estimation of Nitrogen[a] and Tannins[b] in the Protein Fractions of Undefatted Whole Seed Finger Millet Flour

	Purna		EC 4840		HPB 1-8		14-1A-3-26		IE 929	
	N	Tannins	N	Tannins	N	Tannins	N	Tannins	N	Tannins
Fraction A (albumin-globulin)	25.1	Tr	21.8	Tr	21.7	Tr	18.8	Tr	8.7	Tr
Fraction B (prolamin)	38.8	0.02	36.8	0.02	37.4	0.03	34.1	0.03	41.9	0.04
Fraction C (glutelin)	15.2	0.21	14.9	0.17	19.7	0.36	14.7	0.36	15.2	0.74

[a] Percent of total nitrogen of the sample recovered in the fractions. [b] Percent tannins (catechin equivalents).

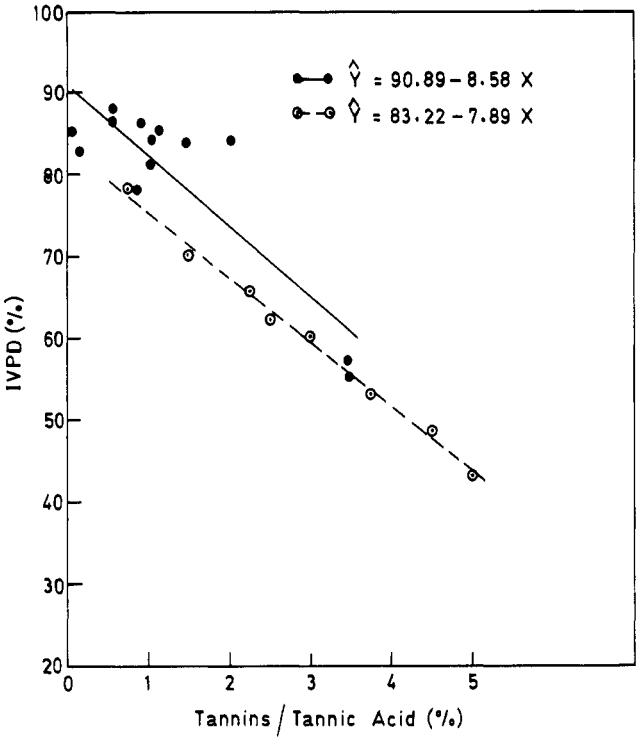

Figure 1. Regression lines of IVPD on tannin levels (closed circles, full line) and IVPD on tannic acid (open circles, broken line) added to finger millet. Equations for the regression lines are also shown.

From the foregoing results it is evident that phenolic and polyphenolic constituents of finger millet do have a deleterious effect on the IVPD of finger millet protein. It was of interest to determine the protein fraction of finger millet with which the tannins were associated. The protein fractions of the nondefatted whole seed flour of finger millet were extracted by a modification of the procedure of Landry and Moureaux described earlier (Virupaksha et al., 1975). Results of the distribution of nitrogen and tannin in the three protein fractions obtained from high tannin varieties of finger millet are summarized in Table VII. The glutelin fraction (fraction C) had the highest tannin content of the protein fractions whereas, the albumin-globulin fraction (fraction A) had only traces of tannins associated with it. The albumin-globulin and glutelin fractions of finger millet have a better distribution of the essential and related amino acids compared with the prolamin fraction (Virupaksha et al., 1975). Association of tannins with the glutelin fraction is likely to affect the digestibility of the glutelin, thereby reducing the nutritive value of the protein of finger millet.

ACKNOWLEDGMENT

The authors thank K. N. Mallanna, Plant Scientist (Millets) for the supply of finger millet samples and N. Sundararaj for assistance in statistical analysis.

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Received for review September 15, 1976. Accepted March 25, 1977.