Tannin Content of Sorghum Varieties and Their Role in Iron Bioavailability

Mukkai R. Radhakrishnan* and Jagarlapudi Sivaprasad

The tannin content of 198 authentic lines of sorghum grain samples—local, high-yielding, and also proven and promising hybrids—obtained from two locations was analyzed. There were wide varietal and locational differences. Local varieties generally contained significantly higher amounts of tannin than did the high-yielding and hybrid varieties. The bioavailability of iron from diets based on high and low tannin sorghum was assessed in normal and anemic subjects. In the 12 normal subjects, absorption of iron from the low and high tannin variety was essentially similar, while in the six anemic subjects, iron absorption from low tannin sorghum was significantly higher. In the other seven anemic subjects, in whom iron absorption was determined after equalizing the phytin content, the difference observed between the two sorghum varieties disappeared, suggesting that at the levels of tannins present in the two varieties of sorghum studied, the tannins had a minor role in determining iron bioavailability.

The millet sorghum (*Sorghum vulgare*), commonly known as jowar in India, is an important food grain for large population groups. The nutritional quality of the protein in sorghum is limited by its low lysine content. The high concentration of leucine in sorghum has been shown to be etiologically related to endemic pellagra seen among sorghum eaters (Gopalan and Srikantia, 1960). Yet another factor of relevance with respect to the nutritional quality of sorghum is the presence in it of polyphenolic compounds referred to as tannins which are known to alter protein digestibility (McGinty, 1969) and produce growth retardation in experimental animals (Jambunathan and Mertz, 1973). Although tannins can complex with divalent metal ions, there is little information as to whether they interfere with the bioavailability of iron. Studies reported earlier from this Institute have shown that iron absorption from sorghum-based diets is low (National Institute of Nutrition Annual Report, 1975). Therefore, an investigation was undertaken to determine the tannin content of varieties of sorghum widely cultivated and consumed in this country. In addition, the availability of iron from diets based on high and low tannin sorghum varieties was assessed in both normal and anemic human subjects.

MATERIALS AND METHODS

One hundred authentic lines of sorghum samples obtained from the All India Coordinated Project for Sorghum, Rajendranagar, Hyderabad, and 98 grain samples obtained from Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, were examined. These included local varieties, high-yielding varieties, and proven and promising hybrids. Whole grain samples of all these varieties were ground to pass through a 60-mesh screen in a Wiley mill equipped with stainless steel blades. Tannin content was determined in all these ground samples according to the method of Burns (1971) using methyl alcohol extraction and vanillin hydrochloride treatment. A standard curve was prepared with catachin (Sigma), and the results were expressed as catachin equivalents.

Iron Absorption Studies in Human Subjects. Two varieties of sorghum with different levels of tannin were selected for this study. The whole grain samples were ground to flour in a local flour mill and was used as such without sieving. The nitrogen content was estimated on duplicate ground samples by the microKjeldahl procedure and the protein content was calculated using 6.25 as the conversion factor. Analysis of phytic acid was carried out on the whole grain ground samples in duplicate following the procedure described by Makower (1970) based on precipitation of ferric phytate and colorimetric determination of iron in ferric phytate after initial conversion to ferric hydroxide. Duplicate samples weighing 2 g of the whole grain ground material was ashed at 500 ± 10 °C and the total ash content was determined. The mineral solution was prepared by dissolving the ash in 2 mL of 6 N hydrochloric acid to a final volume of 25 mL using glass distilled water. Aliquots of the mineral solution in duplicate was estimated for calcium, phosphorus, and iron content by official methods (AOAC, 1960). The chemical composition of the two sorghum varieties used in iron absorption studies in human subjects is given in Table I.

Table I. Composition of the Two Sorghum Varieties Used in Iron Absorption Studies

constituent	low tannin variety	high tannin variety
protein, g/100 g	7.83	9.32
ash, g/100 g	1.58	1.64
tannin, mg/100 g	20	136
total phosphorus, mg/100 g	223	307
phytin phosphorus, mg/100 g	160	273
calcium, mg/100 g	18.7	22.0
iron, mg/100 g	8.7	8.8

Iron absorption studies were carried out using these two varieties on normal subjects in whom the iron absorption was expected to be low and in anemic subjects in whom iron absorption is known to be high. This was done to unmask differences, if any, between the varieties which might otherwise be missed because of the low levels of iron absorption in normal subjects. Since the two varieties of sorghum used differed not only in their tannin content but also in their phytate content, iron absorption studies were conducted using the two varieties of jowar in a group of anemic subjects. The phytin phosphorus and total phosphorus content of the two sorghum varieties was equalized by addition of phytin phosphorus from without in order to examine the extent to which phytin had influenced iron absorption.

Twelve apparently normal healthy adult male subjects from the Institute staff and 13 anemic subjects from the local hospital were selected for these studies. Diets using high and low tannin sorghum ground samples were prepared in the form of a roti and were given with vegetable curry. The roti was prepared as follows.

National Institute of Nutrition, Indian Council of Medical Research, Jamai-Osmania P.O., Hyderabad-500007, India.

0021-8561/80/1428-0055$01.00/0

Table II. Composition of the Sorghum Based Breakfast Used in Iron Absorption Studies

ingredient	quantity
sorghum flour	120 g
potato	25 g
onion	20 g
brinjal	25 g
oil groundnut	2 g
butter	20 g
sugar	15 g
milk	40 mL

Table III. Locational Variation in Tannin Content in Sorghum Varieties

		tannin content in mg/100 g of sorghum variety grown at	
no.	pedigree	Rajendranagar, Hyderabad	Parbhani, Maharashtra
1	555	10	51
2	269	10	73
3	434	10	43
4	2077 B	10	28
5	329	12	65
6	148	12	43
7	CS-3541	15	32
8	604	15	180
9	302	20	145
10	303	27	81
11	563	37	88
12	Swarna	37	198
13	141	58	351
14	3660 B	65	88
15	CSH-4	65	28
16	CK-60 B	69	120
17	CSH-2	100	188
18	CSH-1	170	198
mean		41.2	111.1
SE		9.84	20.05

The weighed quantity of the sorghum flour was made into a dough using water and spiked with either 10 μCi of $^{55}FeCl_3$ (0.1 mg of Fe) or 5 μCi of $^{59}FeCl_3$ (0.1 mg of Fe). The dough was rolled out and roasted on a hot plate (around 200 °C) and prepared in the form of roti (unleavened bread). The vegetable curry was prepared from potato, onion, brinjal, and groundnut oil. The subjects were also given milk with sugar after eating the roti and vegetable curry. This breakfast was given to each subject after an overnight fast. The ingredients of the breakfast and the quantity given are given in Table II. The preparation of the breakfast conform to the usual practice in India and was used in earlier studies from this Institute. Thus each subject received the high tannin sorghum based roti and the low tannin sorghum roti on consecutive days with different radioactive labels. Iron-59 absorption was determined by whole body counting (Narasinga Rao et al., 1972) and ^{55}Fe and ^{59}Fe activities in blood by liquid scintillation counting (Eakins and Brown, 1966).

RESULTS

There were wide variations in the tannin content values ranging from as low as 10 mg to as high as 2056 mg/100 g of the grain. About 50% of the samples had values below 65 mg/100 g and only 5% had values above 400 mg/100 g.

In Table III are given the locational variations in the tannin content of sorghum. The tannin content of varieties grown at Parbhani, Maharashtra, were 2–12-fold higher ($P < 0.01$) as compared to that in the same variety grown at Rajendranagar, Hyderabad.

The data on the tannin content in the grains of local varieties, high yielding varieties and hybrids are given in Table IV. The local varieties had significantly higher amounts of tannin ($P < 0.05$) than did the high yielding varieties. On the other hand, no significant differences were found between the high yielding varieties and the hybrid varieties.

Data on the absorption of iron in normal and anemic subjects from the two sorghum-based diets are presented in Table V. The difference in absorption of iron from the low and high tannin sorghum varieties in the normal subjects was 1.24 ± 0.09 which is not significant. The mean ratio of absorption of iron from low and high tannin diet in the normal subject was 1.47 ± 0.225, and although greater than unity, was not significantly different from one. In the anemic subjects, however, the difference in iron absorption between low and high tannin variety was 5.19 ± 1.76, which is significant ($P < 0.05$). The mean ratio of absorption of iron from low and high tannin diet was 2.14 ± 0.601 which is significantly different from one. In the seven subjects in whom iron absorption studies were carried out after equalizing the total phosphorus and phytin phosphorus content, the mean difference in absorption between low and high tannin variety was 0.83 ± 0.80 which difference is not significant. In these subjects, the ratio of iron absorption from the low and high tannin sorghum was 1.1 ± 0.21 which is not significantly different from one.

DISCUSSION

The tannin content of the sorghum varieties analyzed in the present study may be considered to be generally low, when compared with values reported from Purdue University, where a level of more than 1000 mg/100 g of grain is considered to be high (Jambunathan and Mertz, 1973). Despite the low concentration of tannin in the Indian Sorghum varieties, the total amount of tannin ingested

Table IV. Varietal Differences in Tannin Content of Sorghum

local		high yielding		hybrids	
pedigree	tannin content, mg %	pedigree	tannin content, mg %	pedigree	tannin content, mg %
BP 53	20	148	27	CSH-1	184
Aispuri	87	603	14	CSH-2	144
PJ 16O	87	R-16	15	CSH-3	65
PJ 16K	188	CS-3541	39	CSH-4	46
K local	215	604	97	CSH-5	85
G-3	275	302	20	CSH-6	65
G-4	362	303	54		
		Swarna	117		
		168	48		
		CK 60 B	120		
		370	216		
mean	176		68		98
SE	45.3		18.9		22.0

Table V. Absorption of Iron from Low and High Tannin Containing Sorghum Varieties by Normal and Anemic Human Subjects[a]

expt no.	nutritional status of the subjects	exptl conditions of feeding the diet	no. of subjects	iron absorption, %[b]	difference in iron absorption between low tannin and high tannin	Fe absorption on low tannin / Fe absorption on high tannin[c]
I	normal	low tannin	12	5.05 ± 1.001	1.24 ± 0.91	1.47 ± 0.225
	normal	high tannin	12	3.81 ± 0.900		
II	anemic	low tannin	6	11.49 ± 2.996	5.19 ± 1.76	2.14 ± 0.601
	anemic	high tannin	6	6.29 ± 1.764		
III	anemic	low tannin phytin adjusted	7	3.95 ± 1.96	0.83 ± 0.80	1.10 ± 0.211
	anemic	high tannin phytin adjusted	7	3.10 ± 1.09		

[a] Values given are the mean and SE of the mean. [b] Based on whole body counting of ^{59}Fe activity. [c] Based on the ratio of $^{59}Fe/^{55}Fe$ in blood using liquid scintillation activity.

would be quite large since it is not uncommon for normal adults to consume as much as 400–500 g of the grain in a day, where it is a staple.

Varietal and locational differences in the tannin content of sorghum observed here indicates that varietal differences is a function of the genotype. The locational variation for a given strain of sorghum might suggest that it is not a stable character and that it could be influenced by the nature of the soil, its fertility and climatic factors modifying the phenotype. Similar locational differences in protein content have been reported in sorghum by Deosthale and Mohan (1970), in wheat by Pareira (1944, 1946) and Shollenberger et al. (1949), in barley by Grant and McCalla (1949), and in maize by Curtis and Earle (1946). These variations were explained on the nature and conditions of the soil.

Data presented here on iron absorption studies show that there were wide variations between individuals in the amount of iron absorbed from the low and high tannin containing sorghum diet. In normal subjects although the ratio of iron absorption from the low tannin to that from the high tannin variety tended to be higher than one, it was not significantly different from unity. Such a ratio is indicative of similar absorption rates with the two varieties in normal subjects. On the other hand, in anemic subjects this ratio was significantly higher than one, indicating that the availability of iron from low tannin sorghum was higher than that from the high tannin sorghum variety.

Intestinal absorption of iron is known to be influenced by a number of factors. Important among these is the inhibitory effect of phytates which is present in relatively large amounts in cereals and millets. In the seven anemic subjects in whom iron absorption studies were carried out after equalizing the phytin content, the difference observed earlier between the two varieties of sorghum disappeared. It would thus appear that at levels at which the tannins are present in the two varieties of sorghum studied, they have a minor role in determining iron bioavailability. Since a great majority of sorghum varieties consumed in India have levels of tannin below 130 mg/100 g, this may not be an important consideration in iron bioavailability. However, Diesler et al. (1975) reported that iron absorption was inhibited by tea and these authors postulated that the effect was due to the formation of insoluble iron tannates. Further studies are in progress at this Institute to determine whether relative proportion in which the hydrolyzable and condensed tannins are present in some of the commonly consumed tannin containing foodstuffs of vegetable origin to influence iron absorption.

ACKNOWLEDGMENT

The authors are grateful to S. G. Srikantia, Director, and Bhavani Belavady and B. S. Narasinga Rao, Deputy Directors, National Institute of Nutrition, Hyderabad, for their keen interest and helpful discussions. The authors also thank N. G. P. Rao, Head of the Indian Agricultural Research Institute's Regional Research Station, Rajendranagar, Hyderabad, for providing the seed material for these investigations.

LITERATURE CITED

Association of Official Agricultural Chemists, "Official Methods of Analysis", 9th ed, Washington, DC, 1960.
Burns, R. E., *Agron. J.* **63**, 511 (1971).
Curtis, J. J., Earle, F. R., *Cereal Chem.* **23**, 88 (1946).
Deosthale, Y. G., Mohan, V. S., *Indian J. Agric. Sci.* **40**, 935 (1970).
Diesler, P. B., Lynch, S. R., Charlton, R. W., Turrance, J. D., Bothwell, T. H., Walker, R. B., Mayet, F., *Gut* **16**, 193, (1975).
Eakins, J. D., Brown, D. A., *Int. J. Appl. Radiat. Isot.* **17**, 391 (1966).
Gopalan, C., Srikantia, S. G., *Lancet* 1, 954 (1960).
Grant, M. N., McCalla, A. G., *Can. J. Res.* **27**, 230 (1949).
Jambunathan, R., Mertz, E. T., *J. Agric. Food Chem.* **21**, 692 (1973).
Makower, R. U., *Cereal Chem.* **47**, 288 (1970).
McGinty, D. D., *Proc. Bienn. Int. Grain Sorghum. Res. Util. Conf., 6th*, 20 (1969).
Narasinga Rao, B. S., Prasad, S., Apte, S. V., *Br. J. Haematol.* **22**, 281 (1972).
National Institute of Nutrition, Hyderabad, India. Annual Report, 1975, p 13.
Pareira, A., *Agron. Lusitana* **6**, 367 (1944).
Pareira, A., *Agron. Lusitana* 8, 131 (1946).
Shollenberger, J. H., Curtis, J. J., Jaeger, C. M., Earle, F. R., Bayles, B. B., *U.S. Dep. Agric. Mech. Bull.* 995, 1–33 (1949).

Received for review August 25, 1978. Accepted June 25, 1979.