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POLYPHENOLS IN CEREALS AND LEGUMES

Proceedings of a symposium held during the 36th annual meeting of the Institute of Food Technologists, St. Louis, Missouri, 10 – 13 June 1979

Editor: Joseph H. Hulse

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Treatments of Sorghum Grain That Reduce the Assayable Tannin Content and Their Effect on the Nutritional Value of the Grain[1]

Martin L. Price[2] and Larry G. Butler[2]

A variety of mild alkaline treatments of high-tannin sorghum grain lowered polyphenol content without extracting any components. Marked improvements in weight gain and feed efficiency were obtained by moistening the grain with dilute NH_4OH or 0.5 *M* K_2CO_3. Baking chapaties of high-tannin grain also caused a marked decrease in assayable polyphenol content. However, rats fed a cooked high-tannin grain showed poorer weight gains than did rats fed uncooked high-tannin grain. Rats fed cooked or uncooked low-tannin sorghum performed equally well—significantly better than those fed high-tannin grain.

Japanese sorghum grain importers have been more disturbed than usual this season with imports from Argentina because the tannin content is higher than normal (J. Kishida, personal communication). This was caused by a rainy season near harvest which resulted in substantial losses of all but the high-tannin types (Horacio Pacagnini, personal communication). Approximately two acres of sorghum that we planted in Puerto Rico this spring were wiped out entirely by birds, except for high-tannin types which were scarcely affected. These are two recent, dramatic reminders of the agronomic advantages of growing high-tannin sorghum in regions subject to bird damage or wet weather near harvest. On the other hand, the deleterious nutritional effects of tannin are well known (Price and Butler 1979).

It was found that treatment for several days with ammonium hydroxide, or for an hour with gaseous ammonia under pressure, reduced the assayable tannin in high-tannin sorghum grain by over 90%. Feeding trials with chickens showed that such reductions in tannin were accompanied by improvements in nutritional value, especially for the milder treatments. Table 1 presents 3-week-old chick and 4-week-old rat weight gain on diets composed of BR-54 (high-tannin) or RS-610 (low-tannin) which had been moistened with concentrated ammonium hydroxide at the rate of 10 ml for every gram of whole grain and allowed to stand in a closed container for one week. The grain was subsequently spread in a thin layer to allow ammonia to evaporate and then ground and mixed in the rations. Both weight gain and feed efficiency were markedly improved for the BR-54 by this treatment. It is unclear why the RS-610 was harmed by the treatment.

A different high-tannin grain (Savannah) and RS-610 were treated with dilute ammonium hydroxide prepared by mixing one part of concentrated NH_4OH with six parts of water (same volume/weight ratio as above) for one month at room temperature inside sealed plastic bags. After drying grain for one day at 50 °C, it was ground and mixed in rations. The body weight gains and feed efficiencies of 3-week-old chicks along with tannin analyses are shown in Table 2. Again substantial improvements in the nutritional quality of the high-tannin grain were observed, and this time no significant change was found in the quality of the low-tannin grain. However, the treatment time needed to substantially reduce the assayable tannin was of necessity much longer with dilute than with concentrated ammonia. All of the treatments described so far were on whole grain, which has advantages for drying, storing and transporting the grain.

Subsequent investigations demonstrated that the decrease in assayable tannin could be achieved by a variety of aqueous alkalies, and that grinding the grain decreased the time required for the treatment. Grain (18 kg) was ground, mixed with 3.6 litres of 0.5 *M* K_2CO_3 and kept at 52 °C for 42 hours in sealed plastic bags, then three days later dried as described above. The weight gains of chicks fed rations based on

[1]The research reported has been supported by USAID, Contract No. ta-C-1212.

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Table 1. Three-week-old chick and four-week-old rat feeding trials with sorghum grain (BR-54 and RS-610).[1]

Treatment[2]	% Decrease in assayable tannin	3-week-old chick		4-week-old rat	
		Weight gain[3] (g)	Feed/ gain[3]	Weight gain[3] (g)	Feed/ gain[3]
BR-54					
None	—	163*b*	2.7*b*	36*a*	7.1*a*
NH_4OH	72	247*a*	2.1*a*	63*b*	5.1*a*
RS-610					
None	—	258*a*	1.9*a*	58*a*	5.0*a*
NH_4OH	—	191*b*	2.1*a*	49*a*	5.3*a*

[1]Adapted from Price et al. 1978.
[2]Grain treated for 1 week with conc. NH_4OH.
[3]Values followed by different letters are significantly different ($p < 0.05$).

Table 2. Three-week-old chick feeding trial (Savannah and RS-610).[1]

Treatment[2]	% Tannin	3-week-old chick	
		Weight gain[3] (g)	Feed/ gain[3]
Savannah			
None	2.5	183*a*	2.32*a*
dil. NH_4OH	0.4	268*b*	1.96*b*
RS-610			
None	—	250*b*	1.87*bc*
dil. NH_4OH	—	268*b*	1.82*c*

[1]Adapted from Price et al. 1979.
[2]Grain treated for 30 days with dil. NH_4OH.
[3]Values followed by different letters are significantly different ($p < 0.05$).

Table 3. Three-week-old chick feeding trial with sorghum grain (BR-54 and RS-610).[1]

Treatment[2]	% Decrease in assayable tannin	3-week-old chick	
		Weight gain[3] (g)	Feed/ gain[3]
BR-54			
None	0	174*c*	2.54*a*
K_2CO_3	99	263*a*	2.10*b*
RS-610			
None	—	266*a*	1.84*d*
K_2CO_3	—	218*b*	1.97*c*

[1]Adapted from Price et al. 1979.
[2]Grain treated for 30 days with 0.5 *M* K_2CO_3.
[3]Values followed by different letters are significantly different ($p < 0.05$).

the treated high-tannin grain (BR-54) were substantially improved in comparison with the untreated (Table 3). The control treatment harmed the quality of the low-tannin grain somewhat, but it should be remembered that low-tannin grain would not be treated in practice but only as a control in scientific experiments.

The surprising lability of tannin to a variety of chemical treatments suggested the possibility that some or perhaps even most of the conditions prevailing during cooking sorghum for human consumption might similarly reduce or overcome the harmful nutritional effects of tannin. In many areas of the world where sorghum is used as food for the human population, there is a definite preference for light-coloured and hence low-tannin varieties of sorghum. But these preferences might be overcome in favour of high-tannin grain if the yields of the latter were dramatically higher and more reliable. For the present, no one feels free to recommend the high-tannin route for human consumption because of the likely nutritional consequences. If it could be demonstrated that certain cooking procedures effectively reduced the tannin content, the greatest impediment to their use would be the, perhaps considerable, barrier of human preference.

The original intention of this research was to use one of the common chemical assays to monitor the effect of tannin on cooking, using various recipes. (See Vogel and Graham 1979 for details of many food preparation methods currently in use.) It was found, however, that even moistening ground sorghum to prepare a batter resulted in an apparent loss of much of the tannin. We believe that this "loss" is caused by the formation of an insoluble complex between tannin and protein. Evidence for this view is presented in Fig. 1.

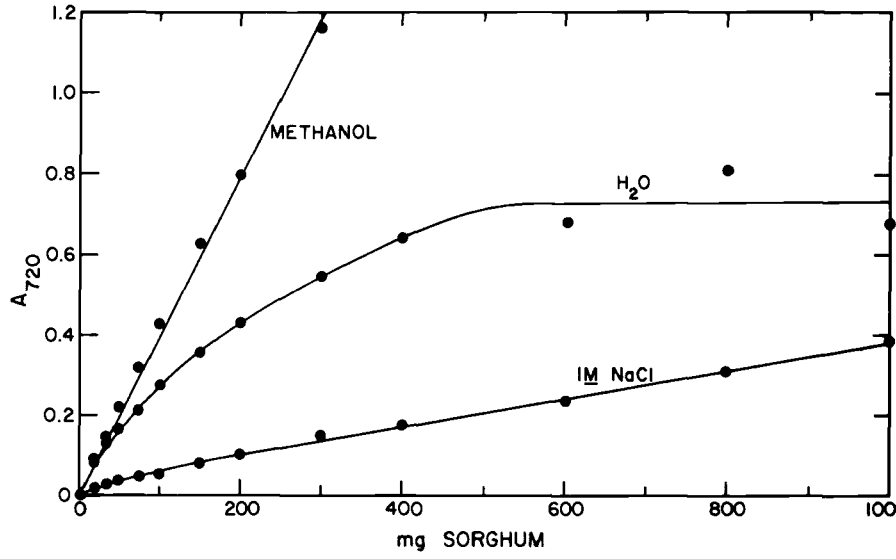

Fig. 1. Absorbance obtained with the Prussian blue assay (Price and Butler 1977) versus mg of sorghum grain per 10 ml of solvent for extraction into methanol, water, or 1.0 M NaCl (adapted from Price et al. 1979).

Capped test tubes containing 10 ml of either water or methanol and varying amounts of ground sorghum grain were rotated for 10 min to extract the tannin. When absorbance due to oxidizable components (tannin and probably other phenols) was plotted against the weight of sorghum in the 10 ml of solvent, a straight line was obtained for methanol. This is consistent with the fact that no insoluble tannin-protein complex formed in that solvent. At very low grain/solvent ratios, water contained nearly as much oxidizable material as did methanol, but this rapidly dropped below the quantities found in methanol as greater amounts of grain were added, and increasing concentrations of tannin and protein were presumably initially attained in the solution.

Price and Butler (1977) presented evidence that by using 1 *M* aqueous NaCl as solvent, the non-tannin oxidizable material could be selectively extracted from sorghum grain. If so, because none of this fraction of the oxidizable material should precipitate protein, it was predicted that a linear relation between absorbance and weight of sorghum would result when salt water was the extractant. This was found to be the case (Fig. 1).

Because it was not possible to monitor "detoxification" of tannin during cooking, it was decided to choose one cooking procedure, followed by feeding trials. Rats were fed diets for four weeks containing only sorghum, vitamins, minerals, and lysine-HCl. The sorghum in one diet had been ground, made into a batter, then baked into thin cakes by cooking 25 min per side in an electric skillet set at 400 °F, dried, and reground. A second diet was based on batter that had been dried at room temperature and reground. A third diet was an untreated control. Both Savannah and RS-610, high- and low-tannin grains, respectively, were tested. Rather than improving the nutritional quality, both treatments caused significant depression in weight gains of rats fed the high-tannin grain (Table 4). None of the treatments affected the low-tannin grain, which gave weight gains significantly higher than the untreated high-tannin grain.

A possible explanation for this decrease in the already low nutritional quality of the treated high-tannin sorghum, while the low-tannin sorghum was unaffected, could be that the tannin complexes with higher quality embryo protein, which is more soluble in water than is the lower quality endosperm protein. Thus the higher quality protein might contribute a disproportionate share to the tannin-protein complex which, if indigestible, would harm the quality of the grain. If whole grain were cooked *before* grinding, the tannins might be forced to complex with protein in the physical vicinity of the testa layer, which is likely to be of lower quality. Very little of the high quality embryo protein should be complexed, and the nutritional quality would be relatively good.

Savannah was boiled 10 min in water, in water containing 0.3% $NaHCO_3$, or in water for 30 min

Table 4. Effect of baking ground grain into chapaties or preparing chapati batter then drying, on measurable tannin content and on rat weight gain and feed efficiency.[1]

Treatment	% Tannin	4-week-old rat	
		Weight gain[2] (g)	Feed/ gain[2]
RS-610			
None	0.0	58.1*a*	4.85*a*
Batter	0.0	56.5*a*	5.12*a*
Chapaties	0.0	58.4*a*	5.17*a*
Savannah			
None	1.9	33.0*b*	8.89*b*
Batter	0.1	20.8*c*	12.7*b*
Chapaties	0.1	17.5*c*	11.8*b*

[1]Taken from Price et al. 1979.
[2]Means followed by different letters are significantly different ($p < 0.05$).

Table 5. Effect of cooking whole grain on measurable tannin content and on rat growth and feed efficiency.[1]

Treatment	% Tannin	4-week-old rat	
		Weight gain[2] (g)	Feed/ gain[2]
RS-610			
None	0.0	70.8*a*	4.83*a*
Savannah			
None	2.2	49.0*b*	5.69*b*
Boiled 10 min in H_2O	1.5	39.8*b*	6.83*b*
Soaked overnight Boiled 30 min in H_2O	0.9	36.4*b*	7.06*b*
Boiled 10 min in 0.3% $NaHCO_3$	0.9	40.1*b*	6.66*b*

[1]Taken from Price et al. 1979.
[2]Means followed by different letters are significantly different ($p < 0.05$).

after having soaked overnight in water, then dried and prepared into diets as described above. Only untreated RS-610 was fed this time, as the point of interest was whether or not Savannah could be improved. Four-week-old rats showed weight gains for Savannah that were significantly lower than for RS-610 (Table 5). All of the treated grains supported still lower weight gains, although differences between the Savannah diets were statistically significant only at $p < 0.1$. This was in spite of considerable decreases in assayable tannin after treatments.

It was then concluded that although various cooking procedures drastically reduce the level of assayable tannin in high-tannin sorghum grain, they do not overcome the deleterious nutritional effects of the tannin. On the contrary, there is evidence that cooking may be uniquely harmful to the nutritional quality of high-tannin grain. The identity of the protein which is complexed with tannin prior to feeding does not seem to be important. Feeding trials need to be conducted on several sorghum varieties before this apparent effect can be considered to be proven.