Tannin Content of Cowpeas, Chickpeas, Pigeon Peas, and Mung Beans

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Ten varieties each of cowpeas (*Vigna unguiculata*), chickpeas (*Cicer arietinum*), pigeon peas (*Cajanus cajan*), and mung beans (*Vigna radiata*) were assayed for condensed tannin content. Tannin concentrations ranged from 0 to 0.7% for cowpeas and 0 to 0.2% for pigeon peas, with essentially no tannin found in chick peas or mung beans. A less extensive survey found no tannin in four varieties of winged bean (*Psophocarpus tetragonobolus*), 0.3% in one variety of adzuki bean (*Vigna angularis*), and 0.1% in Japanese buckwheat. It is concluded that, of the plants surveyed, the cowpea is the only species likely to contain amounts of tannin that may be nutritionally harmful.

Condensed tannins have been reported to occur in some legume species that are important as human food or animal feed, including the seeds of *Phaseolus vulgaris* (common or dry bean) (Ma and Bliss, 1978) and *Vicia faba* (horse, field, or faba bean) (Martin-Tanguy, 1977) and leaves of the forage legumes lespedeza (Jease and Mitchell, 1940), lotus (Jones and Mangan, 1977), crown vetch (Burns, 1963), sainfoin (Jones and Mangan, 1977), and trefoil (Burns, 1963). These findings are of concern to nutritionists and animal scientists because of the deleterious nutritional effects of dietary tannin, as recently reviewed (Price and Butler, 1980).

There are other pulses which are important human foods but which have not been examined for tannin content. This paper reports a survey of the seeds of ten varieties each of four such species: cowpeas (*Vigna unguiculata*), chickpeas (*Cicer arietinum*), pigeon peas (*Cajanus cajan*), and mung beans (*Vigna radiata*). A few varieties of other species, mostly legumes, were also examined, including the winged bean.

EXPERIMENTAL SECTION

Samples were ground on a Udy cyclone sample mill (Tekator, Inc., Boulder, CO) equipped with a vacuum attachment. Extraction of 400 mg of sample was first done in 10 mL of ether to remove material that interferes with the protein precipitation assay, then in 10 mL of methanol for 30 min. Vanillin assays were performed on the methanol extracts by the procedure recommended by Price et al. (1978). The same extracts were used for the protein precipitation assay as described by Hagerman and Butler (1978).

Condensed tannin purified from sorghum grain (Hagerman and Butler, unpublished) was used to prepare standard curves. For both the vanillin assay and the precipitation assay the difference between the absorbance at the appropriate wavelength and the blank (ΔA) was linearly related to the amount of tannin, with correlation coefficients (r^2) of 0.98 and 0.99, respectively. The equations:

vanillin assay:

$$\Delta A_{500} = 0.97 \text{ (mg of tannin)} + 0.03$$

precipitation assay:

$$\Delta A_{510} = 0.72 \text{ (mg of tannin)} + 0.02$$

were used to calculate the percent tannin on a weight basis. It must be noted that because sorghum tannin may not be chemically equivalent to tannin from other sources, the amounts of tannin reported here are not absolute. Bean hull tannin has recently been purified and compared to sorghum tannin (Hagerman and Butler, unpublished).

Samples of cowpeas, chickpeas, pigeon peas, and mung beans representing a broad range of varietal characteristics such as color were provided by the Southern Regional Plant Introduction Station at Experiment, Georgia, from materials grown at that location. Winged beans were obtained from Steve Rice of Purdue University, black gram from W. V. Podhye at Utah State University, and adzuki beans from Leland Hardman at the University of Minnesota. Other seeds were obtained commercially, except for the thornless honeylocust which was grown at Purdue.

RESULTS AND DISCUSSION

The identity, description, and tannin contents of the surveyed seeds are reported in Table I. Excellent agreement was found between the percent tannin as determined by the two independent assays. The correlation coefficient (r^2) between them was 0.99 for all samples which contained at least 0.1% tannin by one of the methods.

The two assays were chosen because they each give unique information about the tannin content. The vanillin assay is specific for the flavan-3-ol and flavan-3,4-diol ring systems found in condensed tannin (Sarkar and Howarth, 1976), but does not distinguish between tannin and its monomeric components. The protein precipitation assay measures only those polyphenols which are able to precipitate protein and not the low-molecular-weight nontannin phenolics. However, this assay does not distinguish between hydrolyzable and condensed tannin. The close correspondence of the two assays in amount of tannin detected suggests that the material measured is largely polymeric and that the tannin is of the condensed (proanthocyanidin) type.

The colorless methanolic extract of most black pulses instantly became purple when the acidic solution was added to measure the absorbance in the absence of vanillin. This would lead to serious overestimation of tannin if background color was measured in methanol without the acid.

The minimum amount of dietary tannin needed to elicit a negative growth response has not been established. Chang and Fuller (1964) found that 0.1% tannic acid (a hydrolyzable tannin) in chick diets had no effect, but that 0.5 and 2.0% caused 7-week growth depressions of 3 and 32%, respectively. Vohra et al. (1966) found 70% chick mortality with a diet containing 5% tannin acid. Condensed tannin purified from *Vicia faba* seeds and included as 3.9% of the diet caused net weight losses in chicks (Marquardt et al., 1977). Similar responses have been found with rats, but the level of protein in the diet is

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Table I

scientific name	common name	PI[a] no. or variety	color	native source	% tannin: vanillin	% tannin: protein precip
Vigna unguiculata	cowpea	255788	red to orange-brown	Nigeria	0.00	0.00
		271257	red to orange-brown	India	0.00	0.00
		291143	beige	Australia	0.59	0.69
		293474	white	USA	0.00	0.00
		293486	beige	USA	0.20	0.28
		293496	white	USA	0.00	0.00
		293581	grey mottled w/black	USA	0.21	0.33
		339580	white, orange spot	Kenya	0.03	0.07
		339623	dark red	Tanzania	0.13	0.19
		352826	red and white pinto	India	0.00	0.00
Cicer arietinum	chickpea	140291	black	Iran	0.00	0.00
		212092	beige	Afghanistan	0.00	0.00
		251024	beige	Afghanistan	0.00	0.00
		251781	black	USSR	0.00	0.00
		271324	beige	India	0.00	0.00
		292006	light beige	India	0.00	0.00
		315806	pea green	India	0.00	0.00
		315827	brown	India	0.00	0.00
		339278	light beige	Turkey	0.00	0.00
		372596	brown	Iran	0.00	0.00
Cajanus cajan	pigeon pea	394206	red-brown	Iran	0.00	0.17
		394252	dark red-brown	Iran	0.08	0.14
		394768	light tan	Iran	0.00	0.00
		394883	50% tan, 50% grey	Iran	0.00	0.00
		395246	dark red-brown	Iran	0.00	0.00
		395671	red-brown	Iran	0.00	0.00
		396146	red-brown	Iran	0.00	0.00
		397251	red-brown	Iran	0.03	0.08
		397426	red-brown	Iran	0.00	0.00
		397759	red-brown	Iran	0.00	0.09
Vigna radiata	mung bean	256074	pea green	Afghanistan	0.00	0.00
		286297	pea green	Ivory Coast	0.00	0.00
		362321	pea green	India	0.00	0.00
		363188	pea green	India	0.00	0.00
		363555	pea green	India	0.00	0.00
		368278	pea green	USA	0.00	0.00
		376861	pea green	Thailand	0.00	0.00
		376973	pea green	Iran	0.00	0.00
		377234	pea green	Iran	0.00	0.00
		377288	pea green	Iran	0.00	0.00
Psophocarpus tetragonolobus	winged bean	TPT-1	red-brown		0.00	0.00
		TPT-2	red-brown		0.00	0.00
		TPT-6	red-brown		0.00	0.00
		Chimbu	black		0.00	0.00
Phaseolus mungo	black gram		black		0.31	0.45
Phaseolus sp.	dark red kidney bean		dark red	USA	0.00	0.00
	pencil pod black wax bean		black	USA	0.00	0.00
	wren's egg bean		mottled red on beige	USA	0.01	0.00
Vigna angularis	adzuki bean	Takara	red-brown		0.29	0.37
Gleditsia sp.	thornless honeylocust		brown		0.00	0.00
nonlegumes	black Mexican sweet corn		black	Mexico	0.00	0.00
Zea mays	Japanese buckwheat				0.13	0.14
Sorghum bicolor	sorghum	BR-54	brown		1.20	1.50
		Savannah-II	brown		1.20	1.60

[a] PI, plant introduction.

critical (Price and Butler, 1980). Glick and Joslyn (1970) found severe growth depression in rats with 4% tannic acid in diets with limiting protein, but on a high protein (40% casein) diet even 5% tannic acid did not depress growth. The effects of dietary tannin on humans are unknown, although epidemiological considerations have led to the suggestion of a correlation between condensed tannins and esophageal cancer (Morton, 1978).

It is thus unclear what level of tannin would be noticeably harmful to the nutritional value of pulses used as human food. Because pulses are high in protein, it might be expected by analogy to the rat feeding trials that the tannin would be of less significance than, for example, the tannin in sorghum grain. On the other hand, the low total intake of dietary protein common in parts of the world where pulses are a major component of the diet might enhance the effects of tannin.

On the basis of this limited survey of the four legume species studied most thoroughly, only cowpeas are likely to have sufficiently high levels of tannin to be of concern in nutrition. However, all had less than half as much tannin as was found in two representative high-tannin sorghum hybrids. Of the other species studied, only the adzuki bean and black gram have potentially harmful levels of tannin. None of the four winged bean varieties contained tannin. The failure to detect tannin in a few varieties of some species in this survey must not lead to the assumption that all varieties are similarly tannin-free.

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The System K_2O–P_2O_5–SO_3–H_2O at 25 °C

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Measurements of solubility in the system K_2O–P_2O_5–SO_3–H_2O at 25 °C showed that two major solubility zones are described by the double salts $K_4H_5(SO_4)_3PO_4$ and $K_2H_3SO_4PO_4$. These data indicate the most practical conditions for operation of a fertilizer process based on the acidulation of phosphate rock by potassium bisulfate.

The fertilizer industry is continuing the search for the economical production of chlorine-free fertilizer materials containing all three primary plant nutrients. Combining some of the fertilizer processes now being used and some of the solvent purification processes being studied may permit the production of such fertilizers. The salt $KHSO_4$ can be produced at almost zero cost (Pennzoil, 1973) due to the value of anhydrous HCl recovered by the reaction

$$H_2SO_4 + KCl \rightarrow KHSO_4 + HCl \quad (1)$$

and presents numerous possibilities for the production of chloride-free fertilizers. The acidic nature of aqueous solutions of $KHSO_4$ can be used for the dissolution of phosphate rock according to the reaction

$$Ca_{10}(PO_4)_6F_2 + 12KHSO_4 \rightleftarrows 9CaSO_4 + CaF_2 + 3K_2SO_4 + 6KH_2PO_4 \quad (2)$$

This reaction, however, will not go to completion due to the low level of acidity. A more favorable quantity of acid sulfate is indicated by the reaction

$$Ca_{10}(PO_4)_3F_2 + 18KHSO_4 \rightarrow 9CaSO_4 + CaF_2 + 9K_2SO_4 + 6H_3PO_4 \quad (3)$$

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where the excess acid environment is sufficient to dissolve all the phosphate rock. Actually, a value between 12 and 18 mol of $KHSO_4$ appears at first to be indicated; however, the reaction is not this simple. It has been shown (d'Yvoire et al., 1963) that mixtures of K_2SO_4 and H_3PO_4 are not stable but react to form the solid phase $K_2H_3SO_4PO_4$—similar to the ammonium analogue reported in another study (Frazier et al., 1971).

The evaluation of these reactions for the acidulation of phosphate rock has been hampered by inadequate knowledge of the acidic region in the phase system K_2O–P_2O_5–SO_3–H_2O and the composition of the solid phases that control the maximum solution concentration. These solution compositions and solid phase characterizations have now been determined and are described below.

EXPERIMENTAL METHODS

The equilibrating mixtures for this study were prepared from reagent-grade orthophosphoric acid (60% P_2O_5) or superphosphoric acid (80% P_2O_5), sulfuric acid, potassium sulfate, potassium phosphate, and water. The solution compositions were adjusted so that three solid phases were present for the invariant point compositions, two solids for the tie-line compositions, and one solid phase at the system boundaries where the corresponding three-component systems are encountered. Optical microscopy was used to verify that the desired solid phases were present