Evaluation of Several Methods to Determine Tannins in Sorghums with Varying Kernel Characteristics[1]

C. F. EARP, J. O. AKINGBALA, S. H. RING, and L. W. ROONEY,[2] Cereal Quality Laboratory, Department of Soil and Crop Sciences, Texas A&M University, College Station 77843

Cereal Chem. 58(3):234-238

ABSTRACT

Polyphenols (tannins) in 21 sorghum cultivars with differing pericarp color and presence or absence of a pigmented testa were analyzed by seven methods: Prussian blue, vanillin hydrochloric acid (V-HCl) with and without blanks (20-min extraction), modified vanillin hydrochloric acid (MV-HCl) with and without blanks (20-min extraction), MV-HCl (24-hr extraction), and α-amylase inhibition. Subtraction of blanks in the V-HCl and MV-HCl procedures reduced the overall level of polyphenols measured without changing the relative quantities. Significant levels of polyphenols and amylase inhibition were found only in those cultivars with a spreader gene and a phenotypically brown pericarp, whether the pericarp was genetically red or white. When the methods were used to measure polyphenols in the sorghums with a brown pericarp, coefficients of variation (CV) were 9.5, 8.0, 8.2, 11.2, and 13.0% and F values with 9 and 14 degrees of freedom (df) were 105.8, 206.6, 191.5, 149.9, and 72.4 for Prussian blue, V-HCl with blanks, V-HCl without blanks, MV-HCl with blanks, and MV-HCl without blanks, respectively. The CV was 2.7% and the F value with 8 and 7 df was 1735.71 for MV-HCl (24-hr extraction). The CV was 25.3% and the F value with 10 and 21 df was 20.60 for the α-amylase inhibition method. Correlations among methods ranged from 0.85 to 0.99. The MV-HCl (24-hr) had the highest F value and lowest CV but is not recommended because of the long extraction period. The V-HCl method with or without blanks is recommended as the most reliable for analyzing polyphenols in sorghum because they have the next highest F values and lowest CV. The method with the highest F value should be the most sensitive to detecting levels of polyphenols due to genetic variation.

Tannins, polyphenolic compounds that precipitate proteins from aqueous solutions, are found in the pericarp and pigmented testa of some sorghums (Blessin et al 1963). During the past few years, a number of methods have been used to evaluate the relative levels of polyphenolic compounds in sorghum. Some of these have been the vanillin hydrochloric acid (V-HCl) of Burns (1971), the modified vanillin hydrochloric acid (MV-HCl) of Maxson and Rooney (1972), V-HCl with blanks and other modifications (Price et al 1978), the Prussian blue (PB) of Price and Butler (1977), the α-amylase inhibition (Barnes and Blakeney 1974, Davis and Hoseney 1979), the protein precipitation (Hagerman and Butler 1978), and the Folin-Denis (Burns 1963). Much confusion has prevailed because each method is specific for particular group(s) of polyphenols. In addition, the extraction method and treatment of samples affect the analytical values obtained within and among methods. This has made quantitative comparison of tannin levels difficult, if not impossible.

[1] Presented at the AACC 64th Annual Meeting, Washington, DC, October 1979.

[2] Research associate, graduate research assistant, technician, and professor, respectively. Cereal Quality Laboratory, Department of Soil and Crop Sciences, Texas A&M University, College Station 77843.

0009-0352/81/03023405/$03.00/0

©1981 American Association of Cereal Chemists, Inc.

In this study, 21 sorghum cultivars with different pericarp colors and the presence or absence of pigmented testa were analyzed by seven methods and the results were compared. A bleach test was also performed in which the grain was allowed to react with a potassium hydroxide-sodium hypochlorite solution. The purpose of the work reported was to compare several methods of tannin analysis and to suggest a method that gives a reliable indication of polyphenol content in sorghums with different genetic backgrounds.

MATERIALS AND METHODS

Materials

Cultivars of *Sorghum bicolor* (L.) Moench with differing pericarp colors and the presence or absence of the pigmented testa layer were grown at College Station and Lubbock, TX, in 1970–1972. The mature grain was harvested and stored at −4°C until used for analysis. All grain samples were cleaned and then ground in a Udy Cyclone mill to pass a 1.0-mm screen. All samples were stored in screwtop test tubes and analyzed within a week after grinding.

Methods

PB. Samples (60 mg) were extracted at ambient temperatures with 5 ml of H_2O by shaking on a Vortex for 1 min and filtering in a Hirschner funnel. The funnel was rinsed with three 5-ml aliquots of H_2O. An additional 40 ml of H_2O was added to the filtrate, followed by addition of 3 ml of 0.1*N* $FeCl_3$ and 3 ml of 0.008*M* $K_3Fe(CN)_6$. After a 10-min incubation, absorbance was read at 720 nm. A catechin (ICN Pharmaceuticals, Inc., Cleveland, OH 44128) standard curve from 0.0–0.25 mg/60 ml was used in determining tannin levels.

V-HCl. Samples (0.2 g) of ground grain were extracted with 10 ml of methanol for 20 min at 30°C. One milliliter of the resulting extract was reacted with 5 ml of vanillin reagent (50:50 mixture of 1% vanillin/8% HCl in methanol) for 20 min at 30°C, and absorbance was read at 500 nm. For blanks, 4% HCl instead of vanillin reagent was added to the extract, and absorbance was also read at 500 nm. Blank values were subtracted from experimental values to give adjusted data. A catechin standard curve from 0.0–1.0 mg/ml in 0.2-mg increments was used in calculating tannin levels.

M-VHCl. Samples (0.2 g) were extracted with 1% HCl in methanol for 20 min at 30°C with the remaining procedure as described for V-HCl.

M-VHCl (24-hr Extraction). Samples (2.0 g) were shaken on an Eberbach shaker with 50 ml of 1% HCl in methanol for 24 hr at ambient temperature. Then, 1 ml was reacted with 5 ml of vanillin reagent (50:50 mixture of 4% vanillin/8% HCl in methanol) for 20 min and absorbance was read at 500 nm.

α-Amylase Inhibition. Ground grain (0.2 g) was extracted at ambient temperature with 10 ml of H_2O and filtered. A Phadebas tablet (Pharmacia Laboratories, Inc., Piscataway, NJ 18854) was added to 4 ml of the extract. The Phadebas tablet is a water-insoluble, cross-linked starch polymer with a blue dye bound to it. Upon hydrolysis, the blue dye is released in amounts proportionate to the amylase activity. One milliliter of porcine pancreatic α-amylase enzyme, 0.17 units per milliliter (Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178) was added, and samples were incubated for 10 min at 37°C. Reaction was stopped by adding 1.0 ml of 0.5*N* NaOH. After addition of 4 ml of distilled water and filtration, absorbance was read at 620 nm. A standard curve of enzyme units vs absorbance for each lot of Phadebas

TABLE I
Polyphenol Content of 21 Sorghum Cultivars Measured by Seven Methods

					Methods[d] That Measure Catechin Equivalents[e]					
						V-HCl		MV-HCl		
Cultivar	Pericarp Color[a]	Testa[b]	Genotype[c]		PB	With Blanks	Without Blanks	With Blanks	Without Blanks	24-hr	α-Amylase (% inhibition)
SC0172	Red	a	RRYY----		0.08	0.04	0.24	0.03	0.55	0.72	0.6
SC0172	Lemon yellow	a	rrYY----		0.07	0.02	0.10	0.00	0.27	0.37	0.4
SC0172	White	a	--yy----		0.05	0.02	0.08	0.02	0.26	0.33	0.8
SC0112	White	a	--yy----		0.04	0.02	0.05	0.02	0.20	0.33	8.6
Tx09	White	a	RRyyiizz	$b_1b_1B_2B_2ss$	0.07	0.02	0.06	0.02	0.24	0.39	4.0
BT×398	Red	a	RRYYiizz	$b_1b_1B_2B_2SS$	0.07	0.02	0.19	0.02	0.48	0.76	7.4
BT×378	Red	a	RRYYiizz	$b_1b_1B_2B_2SS$	0.09	0.04	0.16	0.03	0.58	0.96	9.6
SC0112	Red	a	RRYY----	$b_1b_1B_2B_2ss$	0.07	0.03	0.08	0.02	0.35	0.53	2.3
SC0112	Red	p	RRYY----	$B_1B_1B_2B_2ss$	0.07	0.04	0.11	0.05	0.50	0.85	4.5
SC0112	White	p	--yy----	$B_1B_1B_2B_2ss$	0.05	0.02	0.09	0.02	0.34	0.68	3.4
SC0064-5-8	White	p	--yy----	$B_1B_1B_2B_2ss$	0.06	0.02	0.10	0.06	0.35	0.85	15.8
D D[f] Feterita	White	p	RRyyiizz	$B_1B_1B_2B_2ss$	0.09	0.03	0.52	0.04	0.88	1.43	4.1
SC0109-14	White	p	RRyy--ZZ		0.07	0.04	0.15	0.19	0.55	1.10	2.4
SC0112	Brown	p	RRYY----	$B_1B_1B_2B_2SS$	0.24	0.88	1.12	0.36	1.00	1.49	26.2
SC0064-5-8	Brown	p	--------	$B_1B_1B_2B_2SS$	0.36	1.52	1.75	0.74	1.19	1.57	38.3
C.[g] Shallu × BT×398[h]	Brown	p	RRYyiiZz	$B_1b_1B_2b_2SS$	0.36	2.12	2.39	0.95	1.51	1.76	18.2
C.[g] Shallu × BT×3197[h]	Brown	p	RRyyiiZz	$B_1b_1B_2b_2SS$	0.32	2.38	2.61	0.97	1.41	1.63	41.6
C.[g] Shallu × BT×378[h]	Brown	p	RRYyiiZz	$B_1b_1B_2b_2SS$	0.34	1.95	2.18	0.94	1.53	1.77	18.3
TX09 × C.[g] Shallu[h]	Brown	p	RRyyiiZz	$B_1b_1B_2b_2Ss$	0.25	1.64	1.81	0.63	1.36	1.55	13.9
SC0166	Brown	p	--------	$B_1B_1B_2B_2SS$	0.99	6.67	7.45	3.48	4.85	5.64	77.0
C.[g] Shallu × D D[f] Feterita[h]	Brown	p	RRyyiiZz	$B_1B_1B_2b_2Ss$	0.26	1.95	2.14	0.77	1.16	1.75	20.4

[a] Visual appearance of the pericarp (phenotype).
[b] a = absent, p = present.
[c] RY = genes for pericarp color, I = intensifier gene, Z = pericarp thickness gene, B_1B_2 = presence of testa, S = spreader gene producing brown pericarp. The genetic information for pericarp color and testa is given to the best of present knowledge (F. R. Miller, personal communication, 1980).
[d] PB = Prussian blue, V-HCl = vanillin hydrochloric, MV-HCl = modified vanillin hydrochloric.
[e] Milligrams of catechin per 100-mg sample.
[f] Doubledwarf.
[g] C. = combine.
[h] Hand emasculations.

tablets was prepared. The percent enzyme inhibition (by tannins) was calculated as follows:

$$\text{Inhibition } \% = \frac{\text{Distilled } H_2O \text{ control, IU/L} - \text{Sample IU/L}}{\text{Distilled } H_2O \text{ control, IU/L}}$$

where

IU/L = International Units of enzyme activity per liter.

Bleach Test. Approximately 10 ml of a 1:5 (w/v) concentration of potassium hydroxide-sodium hypochlorite was added to 30–50 seeds in a test tube. The tube was gently swirled and placed in a 60° C water bath for 7 min. The grain was poured into a tea strainer, rinsed thoroughly, and allowed to dry. Grain was classified visually as dark or light, indicating, respectively, the presence or absence of the testa layer.

RESULTS AND DISCUSSION

A variety of polyphenols are found in sorghum. They differ in hydroxylation and oxidation patterns as well as in molecular size. Condensed tannins of large molecular weight have been associated with lowered feed efficiency in bird-resistant sorghums (Fuller et al 1966, McGinty 1969, Thrasher et al 1975).

Table I lists 21 cultivars of sorghum, with their genotype, if known, for pericarp color and testa, their phenotypic pericarp color, and the presence or absence of a testa layer. The bleach test confirmed that the eight sorghums without a testa and the 13 sorghums with a testa layer were properly classified and were not mixtures.

Results obtained from all the assays except α-amylase inhibition are expressed as catechin equivalents (milligrams of catechin per 100-mg sample), and the α-amylase inhibition is expressed as percent enzyme inhibition compared to a distilled water control. The levels of polyphenols occurring in those sorghums with a brown pericarp and pigmented testa (genotype B_1-B_2-S-) were highest as measured by the V-HCl with and without blanks, MV-HCl with and without blanks, MV-HCl (24-hr extraction), and PB assays. The V-HCl and MV-HCl tests with blanks subtracted gave similar very low values for tannins in the sorghums without pigmented testas.

The vanillin reagent has been shown to react with the flavanols composed of two-ring structures (Fig. 1, A and B) as well as with other polyphenolic compounds such as dihydrochalcones and flavanones (Sarkar and Howarth 1976). The necessary requirement is a single bond between C-2 and C-3. Tannins are condensation products of flavan-3-ols and flavan-3,4-diols; thus, they give positive reaction with vanillin (Gupta and Haslam 1980, Sarkar and Howarth 1976). In sorghums a number of compounds may give a positive vanillin reaction, but these compounds may not be condensed tannins. These other compounds account for the lower

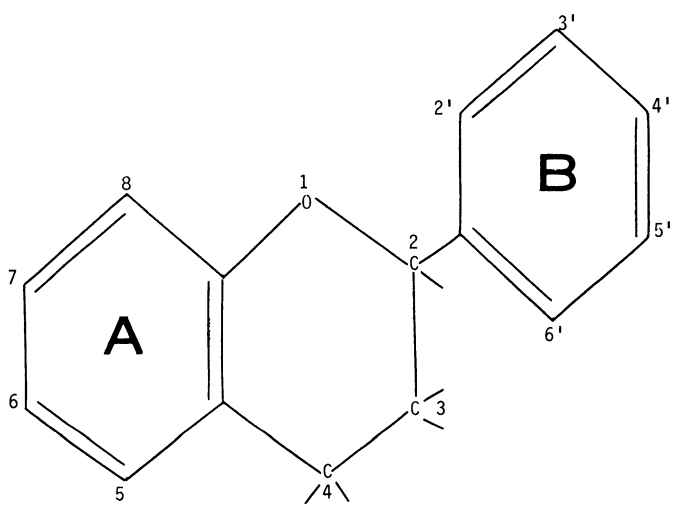

Fig. 1. Basic two-ring (A and B) structure of flavonoid compounds.

levels (less than 1.0 catechin equivalent) of tannin that have been reported for sorghums without a pigmented testa (Butler and Price 1977, Maxson and Rooney 1972). Yasumatsu et al (1965) reported the presence of eriodictyol, a flavanone, in a commercial sorghum. Kambal and Bate-Smith (1976) also reported the presence of the flavanone eriodictyol and the pentahydroxy flavan luteoforol in a white and a red pericarp sorghum, respectively. In the past, these compounds have been reported as tannins. When blanks are subtracted, initial color is removed, but this still does not eliminate the measurement of nontannin, vanillin-positive compounds. Thus, determination of absolute levels of condensed tannin by these methods is not possible; rather, each of these tests is a relative measure of the tannins in sorghum.

In Table II, mean values for the seven methods are presented in three groups: Group I, the eight sorghums without a testa ($b_1b_1B_2$--- or B_1-b_2b_2--); Group II, the five sorghums with a testa but without the spreader gene (B_1-B_2-ss); and Group III, the eight sorghums with a testa and spreader gene (B_1-B_2-S-). When the dominant spreader gene is present, an intense brown pigmentation occurs in the pericarp regardless of its genetic color. The intensity is affected by the relative thickness of the sorghum kernel's mesocarp (determined by the Z gene). Use of the three-group classification scheme was proposed by Cummings and Axtell (1973) based on a comparison of the values measured by the V-HCl and MV-HCl methods. Group I sorghums had catechin equivalents less than 1.0 by both the V-HCl and MV-HCl procedures. Group II sorghums were those with a testa present and values less than 1.0 catechin equivalent with the V-HCl and greater than 2.0 catechin equivalents with the MV-HCl assays. Group III sorghums included those with a testa and with values higher than two catechin equivalents by both methods. Butler and Price (1977) further defined these classifications by using a 20-min extraction time rather than 24 hr and by subtracting blanks from experimental values to reduce background color. Group I sorghums had little or no detectable tannins by either method. Group II sorghums produced very low values by the V-HCl assay and moderately high values by the MV-HCl assay. Group III sorghums gave similar values by both methods. Butler and Price (1977) have postulated that the higher values for Group II sorghums by the MV-HCl method are the result of a covalent attachment of the tannin to some component in the kernel. The acidic methanol extract seems to increase the rate of extraction, resulting in higher values. This would be expected if the rate-limiting step was acid-catalyzed hydrolysis (Butler and Price 1977). Futher modifications in the V-HCl and MV-HCl assays were recommended by Price et al (1978). The first recommendation was for a 20-min extraction period. In the MV-HCl assay the tannin values were much lower at 24 hr than at 20 min, indicating tannin instability in acidic methanol. To minimize variability due to temperature, they also suggested that the assays be conducted in a 30° C water bath. To decrease temperature dependence and improve linearity of the standard curve, the vanillin concentration was decreased from 2 to 0.5%. The final recommendation by Price et al (1978) was the use of purified tannin rather than catechin as a standard. Differences in reaction kinetics were reported for the two standards. At this time, no source of purified sorghum tannin is readily available. The tannin extracted would also possibly be different for different sorghums. Thus, we recommend obtaining a substantial amount of catechin and storing it in a freezer for future use within a laboratory. A standard curve can then be constructed using the standard catechin concentrations as suggested by Price et al (1978).

Although the MV-HCl method gave values twice as high as the V-HCl method for those sorghums with a testa but lacking a spreader gene (Group II), the average for each was still less than 0.1 catechin equivalent. The sorghums with a testa and the spreader gene present (Group III) gave much higher values for tannins by both the V-HCl and MV-HCl methods, with the V-HCl giving the higher results. The MV-HCl procedure with a 24-hr extraction gave results similar to those of the MV-HCl with a 20-min extraction without blanks subtracted.

Because the grain analyzed had been stored for seven years, data from the MV-HCl procedure with 24-hr extraction (Table II) was

compared to that collected earlier by Maxson on the same samples (Maxson 1973). The mean for Maxson's data for Group I (expressed in catechin equivalents) was 0.45 compared to 0.55 in our data, 1.97 compared to 0.98 for Group II, and 2.29 compared to 2.14 for Group III. The agreement between the values for Groups I and III are remarkably good. The only grains that seem to have been affected by storage are the Group II sorghums. The Group II tannins may possibly be more susceptible to oxidation or other changes during storage than those found in the Group III sorghums. This could be one explanation for the Group II tannin content being lower than would be expected from Butler and Price's (1977) classification scheme.

The quantitative PB method is reported to measure total polyphenols rather than just tannins. In the semiquantitative, colorimetric method, a range of colors is seen among the sorghums, but using the colors as an indicator of nutritional value would be very difficult because compounds other than tannins are measured. In these data, polyphenol levels were low for the sorghums that did not have the spreader gene. Those sorghums with a testa and brown pericarp showed moderately higher levels of polyphenols by the PB method. The PB complex stains glass, which necessitates extra care to clean laboratory glassware.

Subtracting blanks from experimental values reduced the overall levels of tannins reported. As seen in Table II, subtracting blank values more clearly defines the differences between the sorghums with a testa and spreader gene (B_1-B_2-S-) and the other sorghums. The value of subtracting blanks from experimental samples is most readily demonstrated in studies on maturing grain, in which unidentified compounds contribute a significant amount of absorbance to the sample extracts (Blakely 1980). However, for some studies, subtracting the blanks might not add enough accuracy to warrant the extra work, depending upon the goals of the experiments.

With the α-amylase inhibition procedure, only the sorghums with a testa and spreader gene showed significant inhibitory power. Variability was high for this procedure. This variability, combined with the time involved and high cost of the Phadebas tablets, makes the Phadebas α-amylase procedure an undesirable method for routine evaluation of tannin levels in sorghum. Sorghums without the spreader gene had inhibitory levels of approximately 15% or lower.

Analysis of variance was determined for each method based on all 21 samples as well as on the eight brown sorghums (B_1-B_2-S-). In Table III, the F values and coefficients of variation for each method are presented for all 21 sorghums and for the eight brown sorghums. Of the seven methods studied, the MV-HCl (24-hr extraction) assay had the highest F value and lowest coefficient of variation. Disadvantages of this procedure were the length of time required for analysis and the decreased catechin equivalent values compared to those of the 20-min extraction time. The V-HCl method with blanks subtracted had the next highest F value when determined both for the 21 and the eight sorghums. A higher F value indicates that a method is more sensitive in detecting differences in tannin content due to genotype. In the analysis of the eight brown sorghums, the coefficients of variation for the methods ranged from 2.7 to 25.3%. This high variability can be largely

TABLE II
Polyphenol Levels of Three Groups of Sorghum Measured by Seven Methods

	Methods[a] That Measure Catechin Equivalents[b]						
		V-HCl		MV-HCl			
Group	Prussian Blue	With Blanks	Without Blanks	With Blanks	Without Blanks	24-hr	α-Amylase (% inhibition)
I[c]							
Mean	0.07	0.03	0.12	0.02	0.37	0.55	3.7
Range	0.04–0.09	0.01–0.04	0.05–0.24	0.01–0.03	0.20–0.58	0.33–0.96	1.8–15.4
II[d]							
Mean	0.07	0.03	0.19	0.07	0.52	0.98	6.0
Range	0.05–0.09	0.02–0.04	0.09–0.52	0.02–0.19	0.34–0.88	0.68–0.98	2.3–15.8
III[e]							
Mean	0.39	2.39	2.68	1.10	1.75	2.14	31.7
Range	0.24–0.99	0.88–6.67	1.12–7.45	0.36–3.48	1.00–4.85	1.49–4.85	13.9–77.0

[a] V-HCl = vanillin hydrochloric, MV-HCl = modified vanillin hydrochloric.
[b] Milligrams of catechin per 100-mg sample.
[c] Sorghums without a testa; genotypes $b_1b_1B_2$--- or B_1-b_2b_2--.
[d] Testa is present without a dominant spreader gene; genotype B_1-B_2-ss.
[e] Testa and spreader gene are present, and the brown pigments are in the pericarp; genotype B_1-B_2-S-.

TABLE III
F Value and Coefficient of Variation (CV) for Methods[a]

			V-HCl		MV-HCl			
Sorghums Measured	Statistic[b]	Prussian Blue	With Blanks	Without Blanks	With Blanks	Without Blanks	24-hr	α-Amylase
All 21	$F_{22,40}$	203.71	375.3	363.9	186.0	118.7		
	$F_{21,20}$						1,004.7	
	$F_{23,59}$							288.0
	CV	13.3	14.5	13.4	21.5	16.4	6.8	42.3
Eight brown	$F_{22,40}$	105.8	206.6	191.5	149.9	72.4		
	$F_{21,20}$						1,735.7	
	$F_{23,59}$							20.60
	CV	9.5	8.0	8.2	11.2	13.0	2.7	25.3

[a] V-HCl = vanillin hydrochloric, MV-HCl = modified vanillin hydrochloric.
[b] F with numerator and denominator degrees of freedom; CV = SD/sample mean.

TABLE IV
Correlation Coefficients Among Seven Methods[a] of Tannin Analysis

	V-HCl		MV-HCl			
	With Blanks	Without Blanks	With Blanks	Without Blanks	24-hr	α-Amylase
PB	0.98, 0.97[b]	0.98, 0.97	0.98, 0.97	0.98, 0.97	...	0.92,0.89
V-HCl						
With blanks		0.99, 0.99	0.99, 0.99	0.97, 0.98	0.97, 0.99	0.91, 0.86
Without blanks			0.99, 0.99	0.98, 0.98	0.98, 0.99	0.91, 0.86
MV-HCl						
With blanks				0.98, 0.99	...	0.91, 0.86
Without blanks					...	0.89, 0.85
24-hr						0.90, 0.86

[a] V-HCl = vanillin hydrochloric, MV-HCl = modified hydrochloric, PB = Prussian blue.
[b] The first number is for all 21 sorghums; the second is for the eight brown sorghums.

attributed to genetic variation. The brown sorghum SC0166 has a tannin content three times that of the other brown sorghums. If this sorghum is omitted from the analysis of variance, the coefficient of variation for the V-HCl with blanks method drops from 8.0 to 4.4%, indicating a large genetic influence. Considering analysis time, convenience, and reproducibility, the V-HCl method with or without blanks is the most useful method for measuring tannin levels in the sorghums that we have evaluated. We do not feel that subtracting the blanks is essential if one is involved in analyses for a breeding program and only interested in relative levels of tannins. Omitting subtraction of blanks would cut analysis time in half, which would be quite helpful because of the large numbers of samples involved in most breeding programs. Even without blanks subtracted, at least a twofold difference was seen between the Group II and Group III tannin levels in this data. We have found subtracting blanks to be necessary when tannin analyses are performed as part of a study of maturing grain, in which other compounds can interfere with the tannin measurements. In the V-HCl procedures with blanks subtracted, we recommend considering catechin equivalents below 0.1 to be zero or, at best, trace levels rather than absolute measured quantities. These are at the very lowest range of detection and when compared to the sorghums with spreader gene present, the values are very low (100-fold difference) and are insignificant. From this data and that reported by others (Butler and Price 1977), analysis of Group I sorghums by the vanillin procedures seems unnecessary. The presence or absence of a pigmented testa should be observed either by scraping with a knife or by the bleach test. If a pigmented testa is present, then the grain can be analyzed by the V-HCl method.

Correlations between methods (Table IV) were very high, indicating comparable relative tannin levels regardless of method of analysis. No difference was seen in correlations calculated for all 21 sorghums or for the eight brown sorghums.

These studies indicate that selection of one method for use in evaluating the tannin levels in sorghum would be beneficial and would facilitate comparison and exchange of data among laboratories. The V-HCl method with modifications proposed by Butler et al (1978) (20-min extraction, 30°C water bath, and vanillin concentration of 0.5%) appears to be the most promising method for laboratory to laboratory reproducibility. In addition, some confusion could be reduced or eliminated by reporting the tannin level of the sorghums without a testa as trace or unmeasurable levels.

ACKNOWLEDGMENTS

We acknowledge the help of many members of the Cereal Quality Laboratory and the Texas Agricultural Experiment Station's Sorghum Improvement Program in conducting this research; J. W. Johnson, D. T. Rosenow, and F. R. Miller, sorghum breeders, deserve special recognition for providing information on the genetics of pericarp and testa in sorghums. This research was supported in part by Grant AID/DSAN/XII/G-0149 from the Agency for International Development, Washington, DC 20523.

LITERATURE CITED

BARNES, W. C., and BLAKENEY, A. B. 1974. Determination of cereal alpha-amylase using a commercially available dye-labelled substrate. Staerke 26:193.

BLAKELY, M. E. G. 1980. The microscopic examination and chemical evaluation of different grain sorghum genotypes in reference to tannin content. M.S. thesis, Texas A&M University, College Station.

BLESSIN, C. W., VanETTEN, C. H., and DIMLER, R. J. 1963. An examination of anthocyanogens in grain sorghums. Cereal Chem. 40:421.

BURNS, R. E. 1963. Methods of tannin analysis for forage crop evaluation. Tech. Bull. 32. Ga. Agric. Exp. Stn.: Athens, GA.

BURNS, R. E. 1971. Method for estimation of tannin in grain sorghum. Agron. J. 63:511.

BUTLER, L. G., and PRICE, M. L. 1977. Tannin biochemistry progress report. Page 4 in: Annual Report on Inheritance and Improvement of Protein Quality and Content in Sorghum. Report No. 13. Purdue University: West Lafayette, IN.

CUMMINGS, D. P., and AXTELL, J. D. 1973. Relationships of pigmented testa to nutritional quality of sorghum grain in inheritance and improvement of protein quality and content in sorghum. Page 112 in: Research Progress Report. Purdue University: West Lafayette, IN.

DAVIS, A. B., and HOSENEY, R. C. 1979. Grain sorghum condensed tannins. I. Isolation, estimation and selective adsorption by starch. Cereal Chem. 56:310.

FULLER, H. L., POTTER, D. K., and BROWN, A. R. 1966. The feeding value of grain sorghums in relation to the tannin content. Bull. N. S. 176. Ga. Agric. Exp. Stn.: Athens, GA.

GUPTA, R. K., and HASLAM, E. 1980. Vegetable Tannins—Structure and Biosynthesis. Page 15 in: Hulse, J. H., ed. Polyphenols in Cereals and Legumes. International Development Research Centre: Ottawa, Canada.

HAGERMAN, A. E., and BUTLER, L. G. 1978. Protein precipitation method for quantitative determination of tannins. J. Agric. Food Chem. 26:809.

KAMBAL, A. E., and BATE-SMITH, E. C. 1976. A genetic and biochemical study on pericarp pigments in a cross between two cultivars of grain sorghum, *Sorghum bicolor*. Heredity. 37:413.

MAXSON, E. D. 1973. The relation between sorghum tannins and nutritive value of the grain. Ph.D. thesis, Texas A&M University, College Station.

MAXSON, E. D., and ROONEY, L. W. 1972. Two methods of tannin analysis for *Sorghum bicolor* (L.) Moench grain. Crop Sci. 12:253.

McGINTY, D. D. 1969. Variation in digestibility of sorghum varieties. Page 20 in: Proc. Sixth Biennial Int. Grain Sorghum Research and Utilization Conference. Amarillo, TX. Grain Sorghum Producers Assoc.: Lubbock, TX.

PRICE, M. L., and BUTLER, L. G. 1977. Rapid visual estimation and spectraphotometric determination of tannin content of sorghum grain. J. Agric. Food Chem. 25:1268.

PRICE, M. L., and VanSCOYOC, S., and BUTLER, L. G. 1978. A critical evaluation of the vanillin reactions as an assay for tannins in sorghum grain. J. Agric. Food Chem. 26:1214.

SARKAR, S. K., and HOWARTH, R. E. 1976. Specificity of the vanillin test for flavanols. J. Agric. Food Chem. 24:317.

THRASHER, D. M., ICAZA, E. A., LADD, W., III, BAGLEY, C. P., and TIPTON, K. W. 1975. Bird resistant milo for pigs. La. Agric. 19:10.

YASUMATSU, K., NAKAYAMA, T. O. M., and CHICHESTER, C. V. 1965. Flavonoids of sorghum. J. Food Sci. 30:663.

[Received June 19, 1980. Accepted October 23, 1980]