

## COMMUNICATION TO THE EDITOR

### Tannin Content of Sorghum Grain by uv Spectrophotometry<sup>1</sup>

TO THE EDITOR:

Cereal Chem. 55(1): 117-118

Many methods are available for estimation of tannins. Maxson and Rooney (1) evaluated several of those methods and concluded that only three of the methods examined had potential use on sorghum grain. Each method had one or more disadvantages—lack of suitable standard, significant day-to-day variability, and inclination to be time-consuming. Tannic acid of freeze-dried sweet potatoes can be determined by uv spectrophotometry<sup>2</sup>. A modified procedure is described that may have potential use in the estimation of tannin content of sorghum grain.

Hand-cleaned grain of the cultivar Funks G-516, a bird-resistant hybrid sorghum, was ground using a Wiley mill fitted with a 20-mesh delivery tube. A 300-mg sample was weighed into a 50-ml centrifuge tube. After adding 35 ml of HCl-ethanol (20:80, v/v), the centrifuge tube was placed in a shaking 75°C-water bath for 3 hr. The sample was placed in cold water until cool and centrifuged for 10 min at 3000 × *g*. The supernatant was decanted into a 100-ml volumetric flask. The sorghum sample was again mixed with 35 ml of HCl-ethanol and centrifuged

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<sup>1</sup>Published with the approval of the Director of the Arkansas Agricultural Experiment Station.

<sup>2</sup>A. A. Kattan, C. Q. Sharp, and J. T. Burgess. Unpublished data (1966).

as before. The supernatant was added to the 100-ml volumetric flask and brought to volume with HCl-ethanol.

A 5-ml aliquot of the supernatant was diluted to 50-ml volume with 50% ethanol (50 water:50 ethanol, v/v). Absorbance was observed against a 50% ethanol blank at 280 nm using a Coleman Hitachi 101 spectrophotometer. Spectrophotometric scans between 340 and 210 nm resulted in two corresponding maximum absorbance peaks for both the sorghum extract and certified reagent-grade tannic acid (mol wt: 322.2). The peak at 220 nm was that of the solvent, whereas the peak at 280 nm was a measure of both tannic acid and the materials extracted from sorghum. Based on these scans, certified reagent-grade tannic acid was used to prepare analytical standards. A stock standard containing 40 ppm tannic acid in HCl-ethanol was diluted with 50% ethanol to produce analytical standards of 1.6, 2.4, 4.0, 5.6, and 8.0 ppm tannic acid.

Extraction temperatures of 65° and 55° C resulted in a lower amount of tannin extracted than did the 75° C extraction temperature. The extraction temperature of 75° C was considered the maximum allowable temperature as that approached the boiling point of ethanol (78.5° C). The 3-hr extraction at 75° C yielded as much tannin as the 4-hr extraction, but more than either 1- or 2-hr extraction time.

The day-to-day variability for 7 different days was approximately  $\pm 2\%$ . The length of time within which the absorbance of a prepared sample must be observed is very critical in many analytical methods, especially those involving color development. This method did not employ color development; however, sample stability was examined. Absorbance was observed immediately, 1, 2, 3, 4, and 27 hr after sample preparation. Absorbance readings were stable for at least 4 hr after sample preparation.

This method is easy and uses a short extraction time (3 vs. 24 hr) compared to those methods (1) previously determined to have potential use in the estimation of tannin content of sorghum grain.

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[Received July 1, 1977. Accepted September 19, 1977]