

Acid Butanol Assay for Proanthocyanidins

Ann E. Hagerman © 2002

This assay is widely used to determine condensed tannins (proanthocyanidins). Although the assay is simple and gives good indication of the presence of condensed tannins, chemical characteristics of the tannins such as position of the interflavan bond and oxygenation pattern affect color yield significantly.

For example, color yield with quebracho tannin is much lower than color yield with procyanidins such as Sorghum tannin because the interflavan bond in quebracho is not readily broken (Hemingway in *The Chemistry and Significance of Condensed Tannins* (R. W. Hemingway and J. J. Karchesy, eds; Plenum Press) page 98 (1989).

The improvements described by Porter, Hrstich, and Chan, *Phytochemistry* 25, 223-230 (1986) are used here.

Reagents

- Acid butanol: Mix 950 mL of n-butanol with 50 mL concentrated HCl
- Iron reagent: 2% Ferric ammonium sulfate in 2 N HCl. Bring 16.6 mL of concentrated HCl up to 100 mL with distilled water to make 2 N HCl. Dissolve 0.5 g $\text{FeNH}_4(\text{SO}_4)_2 \times 12 \text{H}_2\text{O}$ in 25 mL of 2 N HCl. Store in a dark bottle.

Method

1. In a 13x100 mm screw cap culture tube add 6.0 mL of the acid butanol reagent to a 1.0 mL aliquot of the sample (or a smaller volume of the sample made up to 1.0 mL with the sample solvent).
2. Add 0.2 mL of the iron reagent, and vortex the sample. Cap the tube loosely, and put in a boiling water bath for 50 min.
3. Cool the tube and read the absorbance at 550 nm. Subtract the absorbance of a blank containing only sample solvent, acid butanol and iron from the sample absorbance.
4. Because water decreases the color yield in this reaction, the reaction must be standardized with standard dissolved in exactly the solvent that the samples are to be dissolved in. Nonaqueous samples will give the greatest color, but the assay is sensitive enough to give adequate results even with substantial amounts of water present.
5. Standardize the assay with an appropriate proanthocyanidin, for example 1 mg/mL Sorghum tannin.

Modification for pigments

If the samples are colored before heating (due to flower pigments or chlorophyll) you can read the color before heating and subtract that value from the color after heating [Watterson and Butler, *J. Agric. Food Chem.* 31, 41-45 (1983)]. For chlorophyll, this correction is only approximate because some of the chlorophyll is destroyed upon heating, so the subtracted blank is larger than the actual contribution due to the chlorophyll. Better results can be obtained with the assay incorporating PVP, which also allows determination of leucoanthocyanidins.