

Acid Butanol Assay Modified with PVP

Ann E. Hagerman © 2002

Introduction

See Watterson and Butler *J. Agric. Food Chem.* 31, 41-45 (1983)

This is a method for preventing interference from chlorophyll, which absorbs at 550 nm, from the acid butanol assay. The method is tedious, so should be used only if interference seems to be substantial. The method allows simultaneous determination of leucoanthocyanidins (flavan-3,4-diols and flavan-4-ols), proanthocyanidins and 3-deoxy proanthocyanidins. The absorbance in the acid butanol assay is read before and after heating so both the heat stable anthocyanidins and heat labile 3-deoxy anthocyanidins are measured.

Reagents.

- Insoluble polyvinylpyrrolidone (Polyclar AT) (e.g. Sigma P-6755). Boil the PVP for 10 min in 10% HCl, then decant the fines. Filter and dry the PVP.
- 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. Place about 0.2 g PVP in a 13x100 mm screw cap culture tube. Add 1.0 mL of chlorophyll-containing plant extract. Add 5.0 mL of reagent grade methanol and mix for 5 min.
2. Spin to pellet the PVP (10 min, 2000 x g). Discard the chlorophyll containing supernatant. Repeat this step until the supernatant is colorless-- usually three washings of the PVP.
3. Add 7.0 mL of butanol-HCl to the PVP. Add 0.2 mL of the iron reagent. Agitate on a Lab-Quake mixer at room temperature for 1 hour.
4. Centrifuge.
5. Read A₅₅₀ of the supernatant. Then return the supernatant to the PVP-containing sample tubes (any absorbance at this stage of the assay is due to flavan-4-ols and/or 3-deoxy proanthocyanidins).
6. Heat the tubes at 95 C for 1.5 h with caps loosely screwed on.
7. Cool to room temperature and centrifuge to pellet the PVP.
8. Read A₅₅₀ of the supernatant.

Subtract the absorbance obtained before heating, and compare the absorbances to those of standards. The standards must be run according to this procedure even if they do not contain chlorophyll, because adsorption to PVP alters the color yield of the reaction.