Protein Precipitable Phenolics

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Introduction

This method (Hagerman and Butler, ***J. Agric. Food Chem.*** 26, 809-812, 1978) measures the amount of condensed or hydrolyzable tannin precipitated by a standard protein, bovine serum albumin. The precipitate is dissolved at high pH in the presence of a detergent, and the colored iron-phenolate complex is determined spectrophotometrically.

The method is robust and works well with virtually all plant extracts, although the exact nature of the interaction between protein and tannin affect the assay for each unique plant extract. Both the standard and microscale methods are described here. Even traces of acetone inhibit precipitation of protein by phenolics, so you must remove all acetone from plant extracts before attempting the method.

Reagents

- Buffer A: 0.20 M acetic acid, 0.17 M NaCl, pH adjusted to 4.9 with NaOH (11.4 ml glacial acetic acid, 9.86 g NaCl dissolved in about 800 ml water, then adjust to pH 4.9 with a solution of NaOH, then bring to a final volume of 1 liter).
  To make large volumes of Buffer A conveniently, prepare the following two solutions:
  2 M acetic acid, 1.7 M NaCl. Add 114 mL glacial acetic acid to about 800 mL distilled water, add 99.4 g NaCl, and bring to 1 L with distilled water. Store in refrigerator.
  2 M sodium acetate, 1.7 M NaCl. Add 164.1 g sodium acetate to about 800 mL distilled water. Add 99.4 g NaCl and bring to 1 L with distilled water. Store in refrigerator.
  Buffer A: Mix 40 mL of the acetic acid solution with 60 mL of the sodium acetate solution and bring to 1 L. Check the pH; it should be 4.9.
- BSA: 1 mg/ml bovine serum albumin (Sigma A-6003) in buffer A
- SDS/TEA: 5% (v/v) triethanolamine, 1% (w/v) SDS (50 ml triethanolamine, 10 g SDS brought up to 1 liter with water). Triethanolamine becomes yellow with age, you can use it when it is light yellow but if it becomes brown you should discard and purchase a fresh bottle. Use electrophoresis grade SDS (sodium dodecyl sulfate).
- $FeCl_3$: 0.01 M $FeCl_3$ in 0.01 M HCl. To make 0.01 M HCl, dilute 0.83 mL conc HCl up to 1.00 L with water. Dissolve 1.62 g ferric chloride in 1 L of the acid solution, allow it to sit for several hours. Gravity filter through #1 paper.

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Original Method

1. Dispense 2.00 ml BSA into 15 ml centrifuge tubes or culture tubes that can be centrifuged in a desk top centrifuge.
2. Add 1 ml of alcoholic or aqueous tannin solution or plant extract. The tannin cannot contain any acetone, since even traces of acetone inhibit the precipitation reaction.
3. Mix immediately, and allow sample to sit for 15 min at room temperature (purified tannin) or for 24 h at 4o C (plant extracts).
4. Centrifuge 15 min at 3000 x g (high speed in a desk top centrifuge), pour off supernatant.
5. Redissolve pellet in 4.00 ml SDS/TEA. Add 1.00 ml $FeCl_3$, vortex immediately. Use a Pasteur pipette to help break up the precipitate if necessary, it should be completely redissolved.
6. After about 15 min read absorbance at 510 nm.
7. Subtract an appropriate blank (ferric chloride in SDS/TEA).
8. Standardize with purified tannin from the plant of interest (best) or with purified quebracho tannin or tannic acid.

Scaled down method

1. Use microfuge tubes and 1 mL cuvettes.
2. Prepare the SDS/TEA and ferric chloride reagents as described above.
3. Prepare the BSA solution in buffer A as above, but make it at 5 mg/mL.
4. Prepare the tannin solution at about 0.5 mg/mL in methanol (for Sorghum procyanidin).
5. Mix 50 uL BSA with 250 uL buffer A. Add 100 uL tannin and vortex immediately.
6. Allow to incubate at room temperature for 30 min, then centrifuge for 5 min at 13,000 rpm (max speed on microfuge).
7. Aspirate off the supernatant, then redissolve the pellet in 800 uL of SDS/TEA. The precipitate must be completely redissolved--sometimes the high speed of the microfuge makes the pellets hard to dissolve.
8. Add 200 uL ferric chloride and after 15 min read the absorbance at 510 nm.
9. If the pellets cannot be redissolved after microfuging, then do the assay on this scale but centrifuge in a clinical centrifuge at 3000-5000 rpm to obtain softer pellets.

Ultramicroscale method

This is useful for determining precipitated procyanidin in the radiolabeled protein precipitation method. The precipitate is carefully dissolved in 100 uL of the SDS/triethanolamine solution and then reacted with 50 uL of the $FeCl_3$ reagent. The absorbances are read in a nanoliter scale cuvette.

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