

Extraction and Purification of Sorghum Tannin

Ann E. Hagerman © 2002

Contents

Introduction	1
Reagents.....	1
Method	1

Introduction

This method for purifying tannin from Sorghum grain yields a very high quality procyanidin. The original method (Hagerman and Butler *J. Agric. Food Chem.* 28, 947-952, 1980) included a phenol extraction step to ensure that the product was essentially protein free. However, the phenol extraction step has been omitted from these instructions since it is rarely essential. These instructions can be modified as needed to purify tannin in large quantity from virtually any plant source. Because Sorghum tannin is not commercially available, some workers prefer to purify commercial quebracho tannin for use as an analytical standard.

Reagents

Prepare fresh, because ascorbic acid rapidly oxidizes in solution. It is functioning as an antioxidant during extraction steps.

- Absolute ethanol containing 10 mM ascorbic acid. Dissolve 1.76 g ascorbic acid in 1000 mL ethanol.
- Methanol containing 10 mM ascorbic acid. Dissolve 1.76 g ascorbic acid in 1000 mL methanol.
- 0.05 M acetate pH 4. Dilute 2.85 mL glacial acetic acid with about 800 mL distilled water. Adjust the pH to 4.0 with a concentrated solution of NaOH. Bring the final volume to 1 liter with distilled water.
- 50% acetone. Mix equal volumes of acetone and distilled water.

Method

1. In cold room, grind 200 g dry, mature high tannin (bird resistant) Sorghum grain in Waring blender.
2. Add 600 mL absolute ethanol containing 10 mM ascorbic acid.
3. Stir for 45 min with a stirrer from above; centrifuge (or filter) and discard this extract (contains low molecular weight phenolics).
4. Extract the ground grain 4x with 150 mL methanol containing 10 mM ascorbic acid. Each extraction should be for about 45-60 min, in cold room, stirring from above. After each extraction, the samples are centrifuged and the tannin-containing supernatant is saved.
5. The combined extract should be filtered to clarify if necessary.

6. An equal volume of 0.05 M acetate pH 4 is added to the extract, yielding a cloudy orange solution. The methanol is completely removed by rotary evaporation at 30C.
7. The tannin-containing solution is extracted 3x with 300 mL ethyl acetate. For each extraction, the sample is shaken and the lower (aqueous) phase is saved. The upper layer and the interface are discarded.
8. The sample is rotary evaporated at 30C to a total volume of about 20 mL, and absolute ethanol is then added to make the final sample solvent 80% ethanol.
9. The sample is applied to about 4 volumes of Sephadex LH 20 slurry in a coarse sintered glass funnel. The LH 20 should be equilibrated in 80% ethanol. Use very gentle suction to pull the liquid through the beads; stir the beads gently with a glass rod. You want the tannin to have maximum opportunity to contact and adsorb to the beads. Most of the brown color (tannin) will adsorb to the beads.
10. Wash the beads with absolute or 95% ethanol, gently mixing and then filtering, several times until the eluate no longer absorbs light in the UV. Discard all of this eluate. It may take a fairly large volume of ethanol.
11. Wash the beads with 50% acetone, saving the washes. The washing should again be done slowly and gently.
12. As the tannin is washed off the beads in the acetone, start to rotary evaporate at 30oC to remove acetone from the solution. Combine the washes from the beads. When the washes are no longer dark brown, the amount of tannin being recovered is quite low.
13. Continued washing of the beads should eventually restore them to pure white; they can be stored overnight in 50% acetone to wash away the remainder of the tannin. The beads should then be re-equilibrated in 80% ethanol and stored tightly covered in the refrigerator until next time they are needed.
14. After removing all of the acetone from the tannin, the tannin should be freeze dried and stored in glass vials in a freezer. It can be characterized spectroscopically or by degradative methods.