

Method for Acid Butanol Assay of Dried Fig Samples

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To Determine Extractible Proanthocyanidins

1. Remove fig tissue from the freezer, bring to room temperature, and then weigh approximately 50mg of each sample into a large microfuge tube (1.5 mL). Weighed samples were stored in the freezer for several days before analysis, if necessary.
2. Remove ground fig samples from the freezer and allow them to come to room temperature.
3. Add 500 μ L of 70% Acetone/30% water was added to each fig sample and then cap, Parafilm™ and vortex the microfuge tube.
4. Place tubes on rotary extractor for 30 minutes.
5. Centrifuge fig samples for 5 minutes at 10,000 rpm.
6. Remove each supernatant and placed in a new labeled microfuge tube.
7. Repeat steps 3-6 two more times for a total of 3 extractions. Each extract is placed in a separate tube so the % of total extractible material at each step can be determined.
8. Place 500 μ L of each fig supernatant in another labeled microfuge tube and add 150 μ L of methanol followed by 800 μ L of Acid Butanol and 33 μ L of ferric ammonium sulfate.
9. Heat all samples in a boiling water bath for 10 minutes.
10. Record absorbance of all fig samples at a wavelength of 550nm against a blank comprised of methanol, acid butanol and ferric ammonium sulfate.

To Determine Unextractible Proanthocyanidins in Figs

1. Remove fig tissue from the freezer, bring to room temperature, and then weigh approximately 50mg of each sample into a large Falcon tube (50 mL). Weighed samples were stored in the freezer for several days before analysis, if necessary.
2. Remove ground fig samples from the freezer and allow them to come to room temperature
3. Add 5 mL of 70% Acetone/ 30% water to each sample and cap, Parafilm™, and vortex before placing tubes on the rotary extractor for 15 minutes .
4. Centrifuge samples for 5 minutes at 1800 rpm.
5. The supernatant was removed and discarded (initially was saved and used to confirm large scale gave same results as smaller scale extraction).
6. Repeat steps 3-6 three times.
7. After the third extraction, add 45 mL of acid butanol and 1.8 mL of ferric ammonium sulfate to the solid in the large Falcon tube.
8. Place a small stir bar in each tube and so solid can be stirred while samples are heated in a boiling water bath for 10 minutes
9. Record absorbance of all fig samples at a wavelength of 550nm against acid butanol/ferric ammonium sulfate mixture.

Calculations

1. Absorbance values are converted to ug/uL sorghum procyanidin equivalents using a standard curve generated under the same conditions. The concentration is then adjusted for all dilution steps, so that total ug from the 50 mg sample can be calculated.
2. For the extractible tannins, the % of total tannin recovered at each step is calculated. We confirmed that 80% of the extractible tannin was obtained in the first two extractions, so three extractions were routinely done to obtain extractible tannin.
3. For the unextractible tannins, we assayed the solid after 3x extraction to remove extractible tannins. We made the assumption that anthocyanidin was efficiently produced by solid-bound tannins, and was efficiently released from the solid matrix, during the process.
4. We used the sum of extractible and unextractible tannin to calculate total tannin in the samples.
5. Overall, the error in the analytical method for replicate standards was less than 5%. Replicate determinations of extractible tannin from the same tissue varied by about

Modified 3-21-08 by AEH to re-assay the glasshouse F.

brachylepsis

1. Use about 20 mg dry sample in 50 mL falcon tube
2. Extract 3 x 15 min with 5 mL 70% acetone, rotating
3. Add 45 mL acid butanol, 1.8 mL FAS to solid. Boiling water 10 min, stirring
4. Take 25 uL (1st extract) or 50 uL (2nd, 3rd extracts) and put in 15 mL glass screw top tube. Add 200 uL or 175 uL of methanol, then 800 uL acid butanol and 33 uL FAS. Vortex, boiling water 10 min.
5. Read all abs at 550 nm vs. reagent blank.