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Extraction of Non-Structural Carbohydrates (Total Soluble Sugars + Starch) in Plant Tissues

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[1]Realizing Increased Photosynthetic Efficiency (RIPE)

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Ethanolic extraction of total soluble sugars (liquid fraction) and starch (precipitated fraction) from leaf tissue.

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protocol

Pak S. Chow, Simon M. Landhäusser, A method for routine measurements of total sugar and starch content in woody plant tissues, Tree Physiology, Volume 24, Issue 10, October 2004, Pages 1129–1136, https://doi.org/10.1093/treephys/24.10.1129

Carbohydrates, starch, soluble sugar, extraction, leaf tissue

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Reagents

-Ethanol 80%, ACS grade

*Measure 80 ml of ethanol and 20 ml of distilled water separately and then mix. Do not measure 80 ml of ethanol and then bring to volume.

*80% ethanol is hygroscopic. When opened the ethanol will both evaporate and absorb water over time. Re-use eventually will be at a lower concentration. There is also miscibility of ethanol and water. For example, measuring out 80 mL of ethanol and topping off to 100 mL with water will generate ~75% ethanol. Measuring 80 mL ethanol and 20 mL water separately, then combining them will generate ~95 mL of 80% ethanol.

-Chloroform, ACS grade

-Distilled or MilliQ water

Materials

-100-1000 ul pipette tips or repeat pipettor tips

-2 mL screw cap tubes.

-8 mL tubes, polystyrene round bottom 13 x 100 mm, sterile Falcon 352027

These specific tubes are used because they fit in the UIUC GEGC Speed Vac system. They are not chemical resistant. The resuspension of dried sugars in 1 mL of water NEEDS to be transferred to a polyethylene or polypropylene tube before chloroform cleanup!

Equipment

-1000 ul single channel pipette or repeat pipettor

-Water bath

-Centrifuge

-Speed Vac Concentrator

-Oven, **40 °C**

-Ice bucket

-Optional: Plate shaker

- This protocol uses chemical fume hoods. Understand how to safely and appropriately use a chemical fume hood performing the protocol.

- Chloroform pose serious health risks and ethanol is flammable. Please read all manufacturer safety data sheets before handling. UIUC personnel performing this protocol should be current on "Laboratory Safety", "Chemical Safety- An Introduction", and "Chemical Spills" Division of Research Safety training modules before performing this protocol.

Freeze-dry and grind leaf tissue into a fine powder.

- Freeze-dry according to equipment manufacturer's instructions.
- Freeze-dried leaf tissue can be ground using the tissuelyzer WITHOUT THE USE OF LIQUID NITROGEN.
- Improperly freeze-dried tissue will experience total sugar degradation and significant starch degradation. Improperly freeze-dried leaf tissue will look oxidized and more brown.

Ethanolic Extraction 23m

1 Weigh 10-15 mg of freeze-dried pulverized material into a labeled, 2 mL screw-cap tube. Record the weight.

> Ethanol and the hot water bath incubation will dissolve permanent marker and printer ink on some labels. It is advisable to label both the side of the tube and the lid with a unique id

2 Add **1 mL** of 80% ethanol to each sample. A repeat pipettor is useful for large sample numbers.

3 Vortex to mix the samples until all powder dissolved.

4 Incubate the samples at **80 °C** in water bath for **00:20:00** . 20m

> If using snap-cap tubes, put some weight on top of the samples to avoid the caps opening due to pressure build up from ethanol evaporation. A microtube rack is usually sufficient.
>
> Screw cap lids are OK without added weight.

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5 Centrifuge the tubes at max speed (>15,000 g) for **00:03:00** to precipitate solids. 3m

6 Decant the supernatant into alabeled 8 mL tube.

Usually the pellet sticks, allowing the supernatant to be poured from one tube to another. If the pellet doesn't stick, centrifuge again and use a pipette to remove liquid phase.

7 Repeat steps 2-6 four to six times depending on the tissue/plant. All the supernatants from the same sample should be combined into the correctly labeled 8 mL tube after each centrifugation.

For leaves, four washes is usually enough to remove all the soluble sugars.

8 Store 8 mL supernatant tubes **On ice** or at **4 °C** while tissue is incubating at **80 °C** .

After all supernatants have been combined, ethanolic supernatant can be stored at **-20 °C** for several months.

Total Sugar Resuspension

9 After combining the last ethanolic supernatant, remove the ethanol using a Speed Vac Concencentrator, following manufacturers recommendations.

Thermo-Fisher SPD12 Speed-Vac at 0.1 HPr, **35 °C** , and Thermo-Fisher RVT404 refrigerated vapor trap **-108 °C** . Ensure Speed-Vac is properly balanced.

Ideally samples would be run at **30 °C** but the lowest temperature setting on this instrument was **35 °C** . Warmer temperatures could degrade sugars and damage

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sample integrity.

10 Dry samples on Speed Vac Concentrator just until dry and all traces of ethanol have evaporated.

Using recommended tubes and Thermo-Fisher SPD12 Speed-Vac, it took ~16 hours to remove 4 mLs of ethanol. Recommend loading samples the night before and removing in the afternoon of the following day.

Dried samples can be stored at **-20 °C** for several months.

11 Add **1 mL** of distilled or MilliQ water to the dried sugars in the tube.

12 If using polystyrene 8 mL tubes, transfer the aqueous sugar solution to a polypropylene, polyethylene, or other organic solvent resistant tube.

If polypropylene, polyethylene, or glass 8 mL tubes were used initially, then the following chloroform pigment extraction can be conducted in the current tube (skip to step 13). Organic solvents will melt polystyrene. If a polystyrene 8 mL tube is used, the aqueous sugar solution must be moved to a chemical resistant tube.

12.1 Vortex the tube for 30 seconds. Use a pasteur pipette to pipette the liquid vigourously ensuring all sugars in the tube have dissolved in the water.

12.2 1h

Leave the tubes in an ice bath on a plate shaker for **01:00:00** to help dissolve all the sugar.

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12.3 Transfer the entire aqueous sugar solution to a new labeled chemical resistant tube such as a 2 mL polypropylene or polyethylene microcentrifuge tube.

13 Add 500uL of chloroform.

Prime the pipette tip by filling it with 500 ul chloroform, ejecting the chloroform back into the reagent container, and filling it again.

This will prevent the chloroform from leaking out of the pipette and is only necessary when a fresh pipette tip is used.

14 Vortex.

15 Centrifuge the tubes at max speed (>15,000 g) for **00:01:00** to separate the solvent phases. 1m

16 Transfer only the aqueous phase (upper phase) to a new labeled 2 mL tube.

17 Store at **-20 °C** up to several months.

Starch Precipitate 23m

18 After removing the last ethanolic supernatant, leave the lids off and dry the remaining pellet at **40 °C** overnight

If a **40 °C** oven is not available, samples can be dried at **30 °C** plus **58 °C** for 2-3 hours the following day.

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19 After the pellet is dry, replace the lids. The dried starch pellet can be stored at room temperature.

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