Spectranomics Protocol: Chlorophylls and Carotenoids

Updated: January 11, 2011 **Initials of Last Editor: LCJ**

PROTOCOL: Leaf Chlorophyll and Carotenoid Determinations

(i) **Equipment**

- PerkinElmer Lambda 25 UV-Vis Spectrophotometer
- Talboys High Throughput Homogenizer
- Centrifuge (microfuge)
- Sartorius Balance (0.0001 g)
- Micropipettors (both 0.1-1mL and 1-5mL)

(ii) **Consumable materials**

- 2 ml centrifuge tubes with threaded, rubber sealed O-ring caps
- 15 ml centrifuge tubes
- Chilled acetone
- Magnesium carbonate ($MgCO_3$)
- 5/8" threaded lugs ¼" width, stainless steel and small cone bearings
- Chlorophyll A Standard stock in 100% Acetone
- Micropipette tips (1mL and 5mL capacities)

(iii) **Initial Sample preparation**

- Weigh two leaf disks (total area = 0.7693 cm^2) while keeping other samples cold on dry ice or in liquid nitrogen, with minimum light exposure.
- Place the leaf disks in 2 ml centrifuge tubes prepared with 0.05 mg $MgCO_3$.
- Add two 1/8" coneballs bearing and one threaded lug (screw size: 1/4-20, 3" length- 5/8" long) to each tube.
- Label 15mL centrifuge vials corresponding in number to sample tubes.
- Pipette 3 mL of acetone to each 15mL vial and place in freezer overnight preceding sample preparation.

(iv) **Sample Preparation**

- Fill plastic tub with ice and place sample tubes containing on ice.
- Add 0.75 ml acetone to each sample tube.
- Cap and homogenize for 2 minutes at medium-high intensity in the high throughput homogenizer. Return sample tubes to ice tub.
- Add another 0.75 ml acetone, shake each sample by hand for roughly 5 seconds, then centrifuge for 2 minutes.
- Aliquot 0.75 ml extract into 15 ml centrifuge tube containing 3.0 ml of acetone.
- Centrifuge 15mL vials containing aliquot and acetone in large centrifuge for 5 minutes. Place tubes back in ice tray.

(v) **Measurement procedure**

- Start up the Lambda 25 Spectrophotometer and allow to warm up for 15 minutes. Record start-up time.
- Auto-zero the Spectrophotometer.
- Run three blanks and one chlorophyll-A standard, followed by additional in-house standard solutions (ex. Spinach) in a quartz cuvette to check the instrument.
- Using a quartz cuvette, measure absorbance of the solution at 470, 662, 645, and 710 nm as part of a full scan of the sample (400-800 nm; 960 nm min^{-1} scan speed) using a Perkin Elmer Lambda 25 spectrophotometer.
- If samples have too much sediment (i.e. absorbance reading at 710 nm > 0.05), centrifuge and re-run those samples.
- Rerun blanks and standards every after every thirty samples to maintain accurate readings.
- When finished, shut down the Lambda 25 and record the time used.

(vi) **Data preparation and finalization**

- From the measured absorbance, calculate chlorophyll A, B and bulk carotenoid concentrations on a weight (mg g^{-1}) and area (μg cm^{-2}) basis following the equations of Litchenthaler, H.K. and Buschmann, C. (2001) Current protocols in Food and Analytical Chemistry F4.3.8-F4.2.4 and Lichtenthaler (1987) Chlorophylls and carotenoids – pigments of photosynthetic biomembranes. In Methods in Enzymology Eds. SP Colowick and NO Kaplan) pp350-382. (Academic Press: Sydney):

Equations:
Chl A concentration (μg ml^{-1}) = [11.24*(A662 – A710) – 2.04*(A645-A710)]*dilution factor
Chl B concentration (μg ml^{-1}) = [20.13*(A645-A710) – 4.19*(A662-A710)]*dilution factor
Bulk Carotenoids (μg ml^{-1}) = [(1000*(A470-A710) – 1.90*Chl A_{conc} – 63.14*Chl B_{conc})/214]*dilution factor

Dry weight basis (mg g^{-1} dry leaf) - Concentration (μg ml^{-1}) * Extract Volume (ml) / (Disk dry weight (g)*1000)
Disk dry weight = [Disk wet weight - (disk wet weight *%H2O from total leaf)]; H2O is from bulk leaf sample measured during the field collection.