Grottoli Lipid protocol on Ground coral samples

<https://www.protocols.io/view/extraction-of-total-soluble-lipid-from-ground-cora-bc4qiyvw/dx.doi.org/10.17504/protocols.io.bc4qiyvw>

Preparation of ground samples

1. Grind coral samples into a paste
2. Weigh and sub-sample ground coral paste
   1. 1g of wet paste into freeze-drying vial

Freeze dry ground samples

1. Freeze dry samples
2. Transfer samples into larger glass vials

Day-of lipid extractions

1. Label glass Erlenmeyer flasks & weigh them all
2. Prepare aluminum pans (burn & label)
3. Set up retort stands holding funnels
4. Set up vacuum filter with funnel (250 ml), glass frit base (47 mm diameter), GF/F filter, metal clamp, rubber stopper, disposable test tube, side-arm Erlenmeyer flask
5. Prepare solvents
   1. Chloroform / methanol + butylated hydroxytoluene
6. Prepare KCL (potassium chloride solution

Timed lipid extraction procedure

1. Extract lipids
   1. Add 10mL to extraction vial containing freeze-dried sample
   2. Vortex 30s , let sit for 10mins between each sample
   3. Store in the dark for 1 hr
2. Filter samples
   1. Pour contents of extraction vial through glass vacuum filtration system
   2. Filter for 10 minutes
   3. Dispense 2ml of chlo/meth into cap, vortex and add to filter – Repeat 2x
   4. Move glass filter and particulate matter into a pre-burned aluminum pan for AFDW
   5. Pour liquid into separatory funnel and rinse the test tube
3. Wash the extracted lipid with KCL
   1. Add 4ml of KCL solution to separatory funnel
   2. Gently mix in
4. Drain lipid extract from the separatory funnel
   1. Wash lipids & drain again
5. Dry lipid extracts
   1. They use a drying rig by boiling the flasks, I don’t think we’d need this
6. Weigh lipid samples
   1. Once cooled, weigh them in triplicate
7. The energy content of lipid extracted (in Joules) is:**Weight of lipid extracted (mg) x 39.5 \*** \*Using the enthalpy of combustion for lipid as -39.5 J mg-1 from Gnaiger and Bitterlich (1984)