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The lipid content of the hepatopancreases was determined using the following protocol:

- All weights in the protocol were measured to the nearest 0.00001 g using an analytical balance (Mettler Toledo XS205 Dual Range).

1) Obtain the dry weight of the hepatopancreas. (In order to do this the samples will first need to be dried in a drying oven and the weight of the container should be recorded before the tissue samples are placed inside.)

2) Scrape the hepatopancreas from the sides and bottom of the vial and break apart as much of it as possible with a teasing needle. (A pointer with wood handle works best for breaking the sample apart.)

3) Add HIP (3:2 ratio, this should be made prior to starting the procedure by mixing 3 parts Hexane with 2 parts Isopropanol). Ensure the HIP is well mixed before adding to the sample. **NOTE: ~18ml HIP should be added per 1 gram of sample. This quantity will be added in two separate steps. Find the total volume HIP needed by multiplying the sample weight by 18, and then divide the total volume by two.** Add the first portion of HIP here.

4) Finely grind the sample and the solvent mixture with a flat bottomed glass vial into which a pen has been inserted and secured to act as a handle. When you have finished, rinse the small grinding vial with a small amount of HIP to ensure so that no residue is left on the grinding vial and the entire sample remains in the larger vial.

5) Cap and shake the sample for three minutes at speed seven in the homogenizer (Hard Tissue Homogenizer VWR R30140).

6) Add the second portion of HIP to the sample and shake the sample again for three minutes at speed 7. (Before adding the second portion of HIP make sure it is well mixed.)

7) Place the vial in the ice bath for 10 minutes.

8) Centrifuge vial for 5 minutes at 90% full speed (Centrifuge Damon/IEC Division IEC HN-SII).

9) Transfer all of the liquid from the first vial into a second, clean vial with a cap. There will be solid, un-dissolved tissue remaining in the vial that was just centrifuged. (To ensure that no liquid is lost in this step, it may be best to transfer the liquid using pipettes rather than pouring it from vial to vial.)

10) To this residual tissue (in the original vial) add another 2ml of HIP, to ensure you have extracted as much lipid as possible from the sample. Shake the sample for three minutes at speed seven.

11) Place the sample back into the ice bath for 10 minutes and then centrifuge for five minutes at 90% full speed.

12) Combine the liquid portion here with the first portion. The solid left over is waste and should be placed aside.

13) Add ~5 ml aqueous sodium sulfate (6.67%) to the liquid in the vial. Shake this solution for approximately three minutes at speed seven. **NOTE: The aqueous sodium sulfate is prepared by mixing 1 g anhydrous salt with 15 ml de-ionized water and then shaking vigorously.**

14) Return the sample to the ice bath for 10 minutes and then centrifuge the solution at 90% full speed for five minutes. This should result in two obvious liquid layers.

15) Using a pipette, transfer the top layer into a pre-weighed culture tube. The bottom layer is waste. (This can also be done by removing the bottom layer first and then transferring the liquid. The goal here is to retain the entire top layer without keeping any of the bottom layer.) If you are unsure you have removed the entire waste layer, re-centrifuge for five minutes and remove any more bottom layer that forms.

16) Place the culture tube and remaining solution in a drying oven for at least 48 hours. Before removing the culture tube the solvent should be evaporated completely and an oily, dark residue will be left at the bottom of the tube. (This can be checked by weighing the sample once it appears all of the solvent has dried (tube must cool first) and then placing the sample back in the oven and seeing if it has the same weight after 24 hours has elapsed.)

17) Remove the sample from the drying oven and let the culture tube cool. Weigh it to the nearest 0.0001g. The addition to the weight of the empty tube is the weight of lipids within the original dried hepatopancreas.

*If doing more than one sample at a time, the grinding vial and tools used should be sterilized between samples with methanol, so that one does not contaminate another.