Standard Operation Procedure

Crude Fat – Soxhlet Extraction

1. Scope and Application

- In the proximate system of analysis "crude fat" is taken as the fraction of the sample that is soluble in lipid solvents. This extracted material contains not only the useful lipids but a range of different components, yet such categorization is still important for a broad understanding of the composition of the sample.
- It is an important method in the Weende system of proximal analysis for analysis of fat in food stuffs and animal feed.
- Applicable in general to matrices containing fat, including Faeces.

2. Summary

- The sample is dried and ground into fine particles and is placed in a thimble and into Soxhlet extraction apparatus. In a connected flask, non-polar solvent (e.g.: Petroleum Ether) is heated and evaporated. The solvent condenses and drips into the sample chamber. The condensed solvent permeates the sample and dissolves the fat and drains into the solvent flask. This evaporation and draining process repeats multiple times over a period of 6h. Hence the sample fat is repeatedly extracted into a fixed volume of solvent until all lipid soluble components have been drawn into the solvent.

3. Apparatus

- Extraction Thimble / 24cm Cellulose folded filter paper (grade1),11 microns
- Mortar and pestle
- Soxhlet (24/29 joint)

- Condenser fitted to the Soxhlet
- 250mL round bottomed flask (24/29 joint)
- Cork ring
- Desiccator
- Retort Stand and clamps

- Heating block (Heating mantle, Hot water bath – able to maintain 40-60°C)
- Cooling Unit and water pump (Circulate cold water through the condenser – approx. 15°C)
- Analytical Balance
- Fume Hood
- Oven (can be set at 80°C)
- Rota vapor
- Vacuum grease

3. Interferences

- Careful not to heat sample above 80°C to avoid driving off fatty acids or changing the structure of the fat molecules.

4. Safety Precautions

- Handle petroleum ether with care, petroleum ether is flammable and releases vapors, use fume hood when decanting.
- Always use safety goggles, gloves, vapour respirator and laboratory coat while working in laboratory.
- Wear face shield and protect hands from heat when using the heating mantle.
- Monitor progress of extraction – do not just leave for hours.
- Ensure there is an opening above the condenser to relieve pressure build-up.
- Ensure that no solvent is escaping out of condenser (water in condenser must be cold enough, Hence Check the temperature of the water bath regularly)
- Ensure joints are sealed and glassware is not cracked.
- Dispose used gloves after completion of analysis.
- Clean hands using antiseptic soap.

- Avoid spillage and contact with skin. In the latter case use copious washings with cold water and call for medical attention.

5. Sampling

- *45g wet UD faecal sludge needed per sample (2 replicates)*
- Collect in sealed container.
- Homogenize entire sample.
- Preserve fecal sample (45g) by freeze drying.
- Place in vial and label with name, date and proposed purpose.

6. Sample Preparation

1. Weigh out 5g previously homogenized and freeze-dried sample
2. Grind it with a mortar and pestle.
3. Place in previously defatted thimble (cellulose filter paper: grade 1)

7. Reagents

- Petroleum Ether (40-60°C, evaporation residue ≤ 20mg/L)
- Silicon carbide chips

8. Calibration

Tip

Ensure that the Petroleum Ether is either directly from the original container, or carefully recovered in a clean vessel (with a lid) through distillation.

Standard Curve and Error Estimation

Use medical oil as a standard reference (known value of oil/fat present)

Add 2g defatted sand/silica to thimble, add 0.00625g oil to the sand in thimble, add more defatted sand/ silica to make total weight 5g $\approx 0.125\%$ Crude Fat

Continue for the following:
0.0125g $\approx 0.25\%$
0.025g $\approx 0.5\%$
0.05g $\approx 1\%$
0.075g $\approx 1.5\%$

0.1g ≈ 2%
0.125 ≈ 2.5%
0.15 ≈ 3%
0.175 ≈ 3.5%
0.2 ≈ 4%
Perform Soxhlet extraction on each dilution of the standard oil. Plot a curve of % crude fat determined. Determine the error of the method.

Standard Addition Curve

Add 2g freeze dried and ground UD faeces to thimble, add 0.00625g oil to the UD faeces in thimble, add more UD faeces to make total weight 5g. (Extraction should result in ≈ 0.125% Crude Fat + % Crude fat in faeces)

Continue for the following:
0.0125g ≈ 0.25%
0.025g ≈ 0.5%
0.05g ≈ 1%
0.075g ≈ 1.5%

Plot a curve of % crude fat determined. From this determine the effects of the matrix on the extraction of the standard fat from within the UD faeces (compared to the standard curve without the UD Faeces)

9. Procedure

Set up

- Take the 5g freeze dried (***W1***) and ground sample in the defatted thimble and place it in the Soxhlet chamber.
- Add silicon carbide chips(5) into a 250mL round bottomed flask **A**
- Weigh 250mL round bottomed flask (**A**) to 4 decimal places (***W2***).
- Pour 150mL Petroleum Ether into 250mL round bottomed flask (**A**)
- Connect the 250mL round bottomed flask (**A**) to the Soxhlet apparatus, refer to fig 1 below.
- Ensure 250mL round bottomed flask (**A**), Soxhlet and condenser are connected and joints are greased with vacuum grease.(always grease after adding contents in soxhlet and round bottom flask)

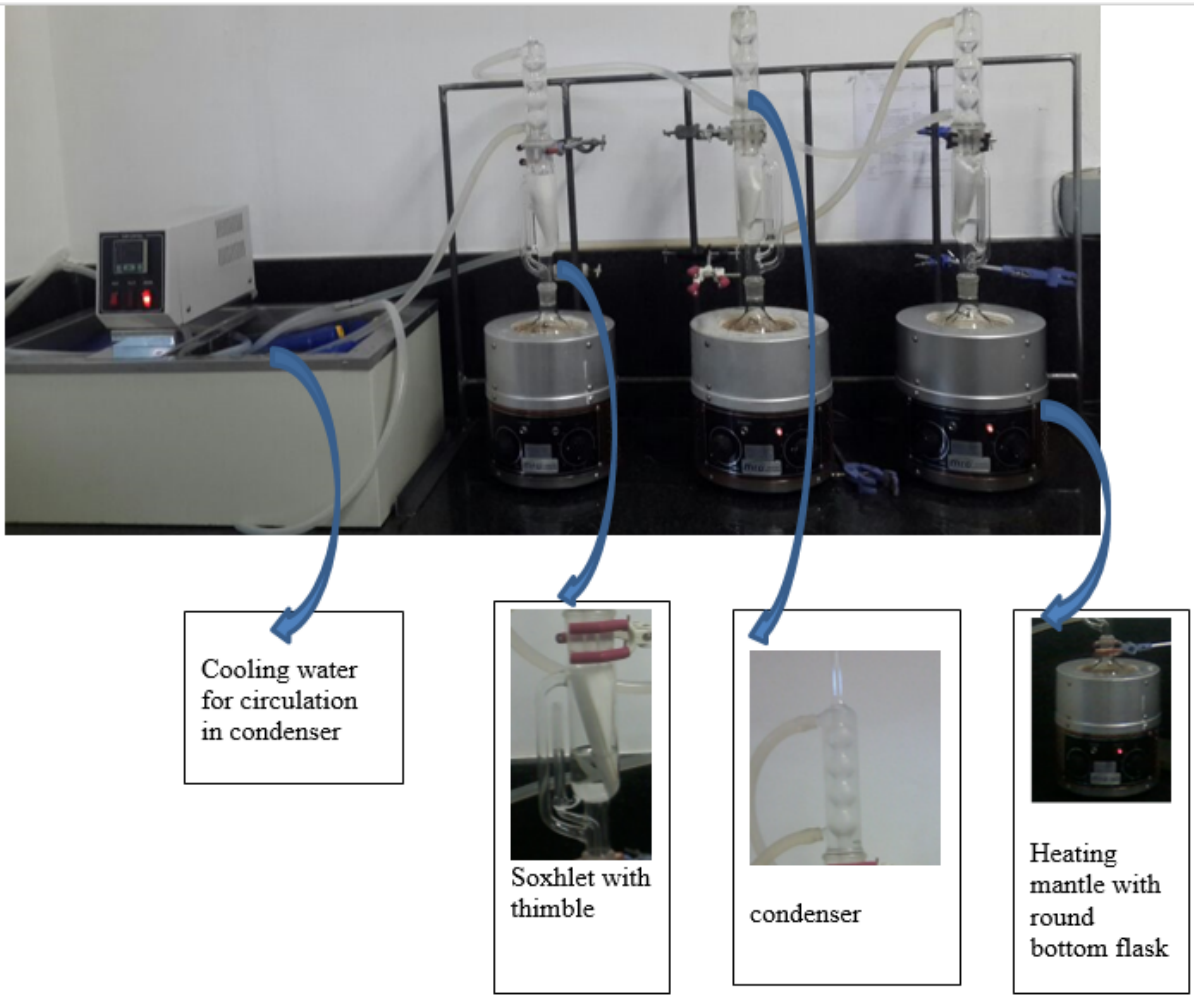
Setup B

- Ensure **Setup B** is securely fastened (retort stand) above a heating mantle, such that the 250mL round bottomed flask (**A**) sits snug in the heating pocket.
- Connect the water cooling system to the condenser and ensure there is a flow of cold water (approx.. 15°C) through the condenser and back into the cooling bath (Condenser: bottom nozzle – water flows in, and top nozzle – water flows out), see picture in fig 1.

Extraction

- Turn the heating mantle on and monitor. Must heat to 40-60°C to ensure the solvent comes to a boil.
- Once the solvent is boiling, ensure that it is also condensing when it reaches the condenser and not escaping out the top of the condenser. The ratio of heat and cooling needs to ensure a closed system.
- Extract for at least 6h, making sure you get 60 siphons.
- Turn off heating mantle and allow 250mL round bottomed flask (**A)** to cool down to approx. room temperature.

FIG 1: SOXHLET SET UP



Open the folded paper and locate the center

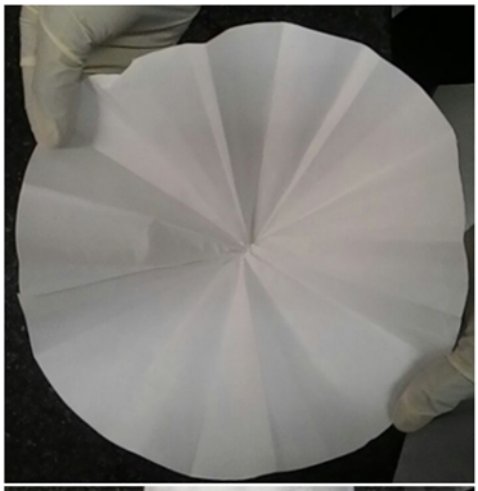
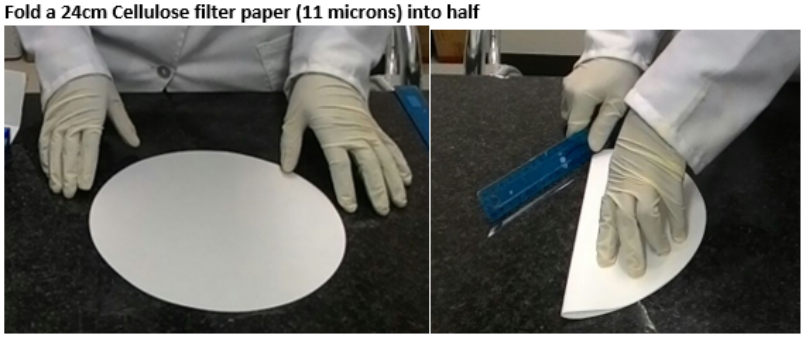


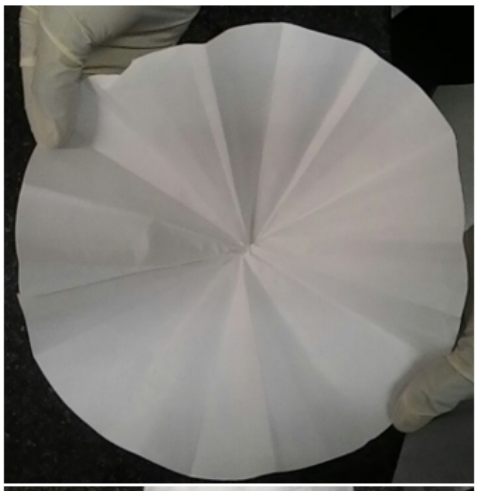
Fig 2; Making a thimble from a filter paper

Fold a 24cm Cellulose filter paper (11 microns) into half

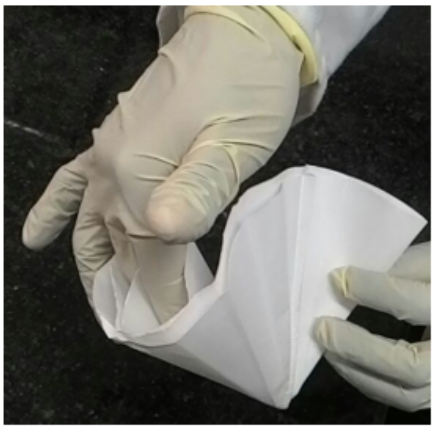


Continue folding in halves until thin

Open the folded paper and locate the center



From the center fold into a cone shape



Drying/Work up Steps

- Remove the 250mL round bottomed flask (**A**) and connect to the rotarevaporater.
- Set rotorevaporator at 350mbar pressure, 60˚C bath temperature and 20 rounds per minute rotation speed when using 40-60˚C petroleum ether as solvent. For 60-80˚C petroleum ether as solvent set pressure at 550mbar, 70˚C bath temperature and 20rpm rotation speed.
- Ensure that the receiving vessel in the rotarevaporater is clean and dry to recover solvent.
- Distil the solvent until 250mL round bottomed (**A**) flask is almost free from solvent.
- Leave 250mL round bottomed (**A**) overnight in a fume hood, to ensure all solvent is evaporated.
- Dry 250mL round bottomed (**A**) with residue for 1.5h in oven set at 80°C.
- Cool in Desiccator.
- Weigh 250mL round bottomed (**A**) with residue to 4 decimal places (***W3***).

Calculation

$$\%\ Crude\ Fat\ (dry\ weight\ basis) = \frac{(W3 - W2) \times 100}{W1}$$

Where

W1 = Initial Dry weight of sample

W2 = Weight of clean 250mL round bottomed flask

W3 = Weight of 250mL round bottomed flask with fat residue

10. References

1. Balthrop, J., B. Brand, R. Cowie, J. Danier, J. de Boever, L. de Jonge, F. Jackson, H. Makkar and C. Piotrowski (2011). Quality assurance for animal feed analysis laboratories, FAO.

APPROVAL OF STANDARD OPERATING PROCEDURE

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