**STANDARD OPERATING PROCEDURE**

**Faculty of Biosciences, NMBU**

**Method name: Total fat**

BIOVIT-no: Arb1052

1. **Introduction**

Determination of total fat using Soxtec™ 8000 Extraction system in combination with Foss Hydrotec™ 8000 Hydrolysis system.

In some cases the fat is chemically or mechanically bound to e.g. carbohydrates, salts or proteins. Acid hydrolysis breaks these bonds – making it possible to extract the fat. For total fat determination the sample is treated under heating with hydrochloric acid. Hydrolysis makes chemically or mechanically bound fats accessible to solvent extraction. The residue is washed and dried and submitted to the following extraction procedure: fat is extracted using light petroleum as a solvent and the Randall modification of the Soxhlet method. The sample is weight into hydrocaps filters and submerged in boiling solvent prior to rinsing in cold solvent, reducing the time needed for extraction. The solvent dissolves fats, oils, pigments and other soluble substances. The extract is then transferred from the hydrocaps to collection aluminum cups. The collection aluminum cups are then placed in a drying cabinet for 30 minutes at 103 °C to evaporate the solvent. The resulting fat residue is determined gravimetrically after drying. This is a fast and straightforward method with low solvent consumption.

**2. Reagents**

* Petroleum ether (boiling point 40-60 °C)
* Hydrochloric acid HCl 3, 4 or 6 N (M), depending on sample type, see respective sample specific Application Note.
* Diatomaceous earth/Celite 566

**3. Risk assessment**

* Petroleum ether:
* Highly flammable
* Avoid skin contact
* Store in a well-ventilated place
* Hydrochloric acid
* Hydrochloric acid is corrosive to the eyes, skin, and mucous membranes.  Acute inhalation exposure may cause coughing, hoarseness, inflammation and ulceration of the respiratory tract, chest pain, and pulmonary edema in humans. Acute oral exposure may cause corrosion of the mucous membranes, esophagus, and stomach, with nausea, vomiting, and diarrhea reported in humans.  Dermal contact may produce severe burns, ulceration, and scarring.
* Store in a cool, dry, ventilated area away from incompatible materials. Store in the original container. Keep containers tightly closed and upright.

**4. Equipment**

* Analytical scale
* Hydrotec™ 8000 hydrolysis unit
* Hydrocaps (240 pcs) TEC 60067520
* Soxtec™ 8000 extraction unit
* Condenser Seal Viton/Butyl 6 set. TEC S800830
* Hydrolysis beaker
* 2 Capsule holders (6 position)
* Tongs
* Weighing Support
* Docking tool for capsules
* Air ventilated oven
* Desiccators
* Cotton pads

**5. Special remarks**

Program Hydrotec™ 8000: 3, 4, 6 M HCl at boiling power 60%.

Program Soxtec™ 8000**:** 100% petroleum ether at 95 ºC.

**6. Sample material**

**Solid samples**

The sample material must be dry, homogeneous and ground to a size of 1 mm or less.

-**Feed samples**

It is recommended to add 1g Diatomaceous earth/Celite 566 to each Hydrocap prior to weighing in the sample. The sand will maintain the sample more porous and results in a more efficient extraction.

**Semi solid samples and pastes**

This category of sample is the most difficult to handle particularly when there is a wide variation in particle size and/or hardness of constituents. Depending on the sample type, homogenizing or ball milling may provide a suitable sample for analysis.

**Liquid samples**

The general recommendation for liquid food samples is to add 1 g Celite 566 to the Hydrocap prior to weighing the sample in.

**Sample Weight**

To weigh samples for fat analysis an analytical balance accurate to 0.1 mg should be used. The actual sample weight should never exceed what is given in the method, this to ensure proper reagent activity during the treatment, see Table 1.

Tabell 1: Sample weight

|  |  |
| --- | --- |
| **Sample weight recommendation** |  |
| **Fat content** | **Sample weight** |
| 0-10 % | 2-3 g ±0.1 mg |
| 10-25 % | 1-2 g ±0.1 mg |
| >20-25 % | 0.5-1 g ±0.1mg |

**7. Work procedure (Hydrotec™ 8000)**

**Reagents:** 3, 4, 6 M HCl and Petroleum Ether 40-60%. Consumption of the HCl is about 1.8 liters per hydrolysis.

1. Using the thimble support on the scale, weigh the samples into the hydrocaps, record the weight, and place them into the plastic holders. Max 6x2 samples- these are clicked together twice to fit into the beaker.
2. Mark the aluminum cups well and weigh them in. Record the weight.
3. Load the hydrocaps into the beaker.
4. Lower the lid, and secure that it seals against the beaker.
5. Replace the hydrotec fumes filter.
6. Check that the cooling and rinsing water taps are fully open.
7. Check the HCL tank, minimum 1.8 L (under the fume hood).
8. Power ON.
9. Select the program 1 (60 % - 0,45 min - 1,45-OFF – 10 - OFF).and press start  button (Automatic hydrolysis and wash).
10. During boiling, check and if needed adjust the boiling power.
11. When the hydrolysis is finished, lift the lid.
12. Check that the pH is neutral after the last rinse (once a week). Use the litmus paper (pH> 6.2 is optimal).
13. pH can be measured directly from wastewater. If the waste tubing’s are lifted up when the waste is pumped out, waste will return in the beaker with the samples.
14. Take out the beaker.
15. Take out the capsule holders, using the tongs.
16. Wipe down any residue on the inside of the hydrocap (the upper part) with a piece of cotton. Place the cotton inside the hydrocap on the top of the sample.
17. Dry hydrocaps in an air ventilated oven 60 °C overnight.
18. Prepare for the extraction.

**Work procedure (SoxtecTM 8000)**

**Reagents:** Use 2/3 of Petroleum Ether from Recovery and 1/3 of new Petroleum Ether 40-60%.

1. Aluminum cups are washed and dried at 103 ° C for 30 minutes and cooled in a desiccator.
2. Mark the aluminum cups well and weigh them in. Register the weight.
3. Check that the solvent recovery flask is empty. If there is some liquid there, the procedure will not start.
4. Transfer the thimbles to the to the thimble stand or to the thimble docking tool.
5. Turn the instrument on. Answer questions by pressing OK. Wait until the instrument has stabilized.
6. Open the water tap.
7. Press the sample down, push the button and insert the thimble docking tool to attach all the thimbles to the sample holders. Press the sample up by using the push button and pull out the docking tool.
8. Remove the sample holder.
9. Insert the cup holder with the collection/aluminum cups. Press the condenser down and push the button to dock the collection/aluminum cups onto the hotplate.
10. Close the front glass.
11. Using the wheel with 0 and 6 positions, on the left side of the instrument, add 85 ml of solvent to all of the samples - when the addition is complete set the wheel to 0.
12. When not running 12 samples, open the menu, plus program, position, and change (from ON to OFF). Then, you exclude the positions where there is no sample in. If you only run a few samples, they are distributed beyond the two soxtecs.
13. Choose the program.
14. Program 1 for "normal extraction" (Table 2).
15. Press start push button on the control unit. Extraction program is carried out automatically.
16. When the extraction is completed (sound signal), remove the cup holder. The cups are quite warm, use a pliers and put the cups into the round holders customized to air ventilated own.
17. Place the cup in an air ventilated oven (103 °C) for 30 minutes.
18. The cups are brought over in a desiccator to cool. (approx. 60 minutes).
19. Weigh the cups and calculate the fat % in a sample.
20. Insert the thimble docking tool to remove all the cellulose thimbles from the sample holders.
21. Hydrocap filters are used only once.

**8. Calculation**

g fat/kg sample = (Weight tube w/fat – weight tube) \* 1000

Sample

Where:

Weight glass w/fat = weight of collecting pipe with fat (g)

Weight glass = weight of empty collection pipe (g)

1000 = g/kg

sample = gram weighed sample in the cell (g)

Present as % or g/100g.

**History - instrument transitions and method modifications**

Instrument transition 2021: from ASE® 350 Accelerated Solvent Extractor (Dionex, USA) to

SoxtecTM 8000- FOSS analytical.