**STANDARD OPERATING PROCEDURE**

**Faculty of Biosciences, NMBU**

**Method name: Crude fat**

BIOVIT-no: Arb1045

1. **Introduction**

The SoxtecTM 8000 performs crude fat extraction from a wide range of sample types such as feed, cereals, meat, fish, dairy products and other food products.

Fat is extracted using light petroleum as a solvent and the Randall modification of the Soxhlet

method. The sample is weight into cellulose thimbles 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 cellulose thimbles 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)
* Diatomaceous earth/Celite 566

**3. Risk assessment**

* Petroleum ether:
* Highly flammable
* Avoid skin contact
* Store in a well-ventilated place

**4. Equipment**

* Analytical scale
* Soxtec™ 8000 extraction unit
* Cellulose thimbles 33x80 mm 25/PAC TEC 15220045
* Condenser Seal Viton/Butyl 6 set. TEC S800830
* 2 Capsule holders (6 position)
* Tongs
* Weighing Support
* Docking tool for capsules
* Air ventilated oven
* Desiccators
* Cotton pads

**5. Special remarks**

Program 1. Soxtec™ 8000**:** 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.

* **Semi solid samples and pastes**

Depending on the particular sample type, homogenizing or ball milling may provide a suitable sample for analysis. Samples rich in fat and moisture that have been homogenized need to be further prepared before extraction. There are many variations to this treatment, the following is recommended for this class of sample:

• Put sand or any other inert filter aid material such as Celite 566 or diatomaceous earth in the bottom of the extraction thimble and weigh the paste directly into the thimble.

• Mix the sample with the sand/Celite using a glass rod. After mixing, wipe the rod off with a

small piece of cotton and put this inside the thimble.

• Place a thin wad of defatted cotton on top of the sample and press it down into the center. A

“cotton channel” in the center of the sample is formed.

• Dry the mixture in the thimbles in an oven for 2 hours at 103 ±2 ºC, if nothing else is

recommended in the sample specific Application Note.

* **Liquid samples**

Depending on what is to be extracted from the sample, different procedures will be needed.

Samples containing particles, like wastewater, are filtered to collect the parts that will be extracted. For suspensions adsorption on inert material such as Celite silica or cotton could be the choice. For smaller volumes (10-20 ml) or when the extractable matter cannot be separated by filtration, two other procedures are possible:

• If the extractable matter is not volatile, vacuum drying may be tried.

• Absorption of the liquid using Celite or cotton.

**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 (SoxtecTM 8000)**

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

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. Attach the thimble adapters on the thimbles. (When using the cellulose filter for the first time).
5. Using the thimble support on the balance, weigh the samples into the cellulose thimbles, record the weight and then transfer the thimbles to the to the thimble stand or to the thimble docking tool.
6. Turn the instrument on. Answer questions by pressing OK. Wait until the instrument has stabilized.
7. Open the water tap.
8. 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.
9. Remove the sample holder.
10. 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.
11. Close the front glass.
12. 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.
13. 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.
14. Choose the program.
15. Program 1 for "normal extraction" (Table 2).
16. Press start push button on the control unit. Extraction program is carried out automatically.
17. 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.
18. Place the cup in an air ventilated oven (103 °C) for 30 minutes.
19. The cups are brought over in a desiccator to cool (approx. 60 minutes).
20. Weigh the cups and calculate the fat % in a sample.
21. Insert the thimble docking tool to remove all the cellulose thimbles from the sample holders.
22. Clean the cellulose thimbles using a brush. Cellulose thimbles will be used again.
23. It is best, after a certain time, to run recovery test on cellulose thimbles.

**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

### [Soxtec™ 8000 - FOSS analytical.](https://www.fossanalytics.com/en/products/soxtec-8000) .