Customs Laboratories
European Network

ILIADe 179:2023 | CLEN Method

Determination of Total Fat Content in Food Products

Version 11 December 2023

This table shows the most important changes that have been made compared with the latest former version	
Date of the latest former version: 14 October 2019	
Section	**Changes**
5.	In 5.1: not compulsory to use anhydrous sodium sulphate. In 5.2: correction 0.2% (in the sentence "relative percentage difference between two subsequent weightings should be ≤ 0.2%") and addition of two possible cases for the calculations.
8.	Confirmation of the precision data after the CLEN 9th Meursing proficiency test performed in 2021 (precision data identical to 14 October 2019 version).

Determination of Total Fat Content in Food Products

1. Scope

The value of fat content obtained with this procedure shall be used in the calculation of milk fat content required for the attribution of Meursing additional code (method described in Regulation (EC) No. 900/2008,modified by Regulation (EU) No. 2015/824).
This method can be applied for the analysis of food, where oils and fats cannot be extracted without a previous hydrolysis, except for the case where specific methods apply, such as milk, butter or cheese.
It has been tested also on nutritional supplements containing skimmed milk together with egg white powder and thickeners such as carrageenan and xanthan gum. In these cases results should be carefully regarded, as for “fat content” in this procedure is meant all substances extractable with this procedure.

2. Principle

The sample is hydrolysed in a hot cup with hydrochloric acid. It is then accurately filtered together with a filter aid that should retain all the fat. After washing and drying, the residue is extracted by petroleum ether in a Soxhlet extractor or equivalent system, and weighed.

3. Reagents and materials

3.1. Hydrochloric acid 4 N.

3.2. Filtration system: it is composed of a filter and a filtration aid, an auxiliary material, which mainly serves in the filtration process to confer porosity and incompressibility of the solid deposit that accumulates in the filtration medium, aiding the retention of the fatty matter. The choice of the filters should be done finding the best compromise between loose of fat and speed of filtration while filtration aid has to be chosen in order to retain all the fat during filtration and totally release it during Soxhlet extraction.
Suggestions may be:

- cellulose filters for rapid filtration plus diatomaceous earth treated with sodium carbonate and calcined, trade name Celite of the type 503 or similar;
- glass fibre membrane filtres plus cellulose (microcrystalline; ca. 0.038 mm);
- glass filter discs filled with quartz sand (40 - 100 mesh) or ca.50 g quartz sand (0,3-0,9 mm)and ca.5 g Celite type 545.

3.3. Anhydrous Sodium sulphate.

3.4. Petroleum ether (40°C – 60°C), with non-volatile substances less than 0.001 % m/m.

3.5. Cellulose thimbles or glass thimbles or glass tubes.

4. Apparatus

4.1. Analytical balance with precision of 0.1 mg.

4.2. Desiccator provided with a perforated plate for rapid cooling, containing active drying material.

4.3. Ventilated oven (better if with vapours sucking, to remove ether).

4.4. Hydrolysis dedicated equipment: hydrolysis system or heating mantles/plates equipped with a reflux system, or another adapted heating system.

4.5. Soxhlet extractor or equivalent extraction system (manual, semi-automatic or automatic).

5. Procedure

The sample should be finely homogenised in a mortar or through a blender. Weigh in a 250 ml flask or a beaker a quantity of sample not less than 1 g. The ideal is to weigh a sample quantity as to have a TFC of approximately 1 g (Sample Amount to be weighed = 100 / percentage of presumed TFC). The limiting factor is, however, the maximum quantity of sample in order not to have problems of incomplete extraction.

Different procedures can be followed to perform both the acid hydrolysis and Soxhlet or equivalent extraction. Conditions such as quantity and/or concentration of HCl, temperature or heating time for hydrolysis, drying duration and temperature, duration and conditions for extraction and for final drying of extract must be clearly defined in in-house documents and tested for satisfactory recovery of fat (98%) on certified materials or within the specifications of the certified reference materials used to check the accuracy of the method.

Procedures indicated in this document are general suggestions.

5.1. Acid hydrolysis

For example, acid hydrolysis can be performed by addition of 4 N HCl normally in a flask or a beaker at boiling temperature (it can be under reflux or on a hot plate or in a dedicated equipment), leaving it for at least 30 minutes. Filter through filter system and wash the filter several times with deionized water until neutral pH of filtrate. Introduce the filter in an extraction thimble and dry it (for example in a drying oven). Before extraction a small amount of anhydrous sodium sulphate may be added.

5.2. Soxhlet (or equivalent) extraction

Weigh with precision of 0.0001 g the extraction glass or collection flask (depending on whether a Soxhlet or equivalent extraction system is used or a manual extraction is performed) after reaching a stable weight (treating in ventilated oven and cooling in a desiccator) Place extractor glasses or collection flask and extraction thimbles in the Soxhlet system and perform the extraction with petroleum ether and final drying of extractor glass/collection flask with usual procedure, allow to cool in the desiccator at room temperature and weigh to the nearest 1 mg, provided constant mass was obtained (relative percentage difference between two subsequent weightings should be ≤-0.2%).

with:

W^{II} = weight of extraction glass or collection flask after half an hour treatment in oven (in grams)

W^{I} = Previous weight of extraction glass or collection flask (in grams)

Av = average between W^{II} and W^{I}

Case 1: $W_{II} > W_I$ --> constant weight --> $Av = W_I$

Case 2: $W_{II} < W_I$ $\quad \text{Relative percentage difference } \% = \frac{W^{I} - W^{II}}{Av} * 100$

Blank test should not exceed 0.01 g for manual and 0.005 g for automatic extraction.

6. Calculation

Total fat content in sample, expressed in % m/m, is given by the following formula:

$$TFC = \frac{A - B}{C} \cdot 100$$

where:

A = final weight of recipient containing extracted fat (in grams).

B = initial weight of empty recipient (in grams).

C = sample weight (in grams).

7. Expression of results

Results are given with one decimal place (i.e. a maximum of 3 significant digits, with one decimal).

8. Precision

Ten CLEN inter laboratory tests were carried out between 2002 and 2023, including the determination of total fat content, with the participation of the EU Customs laboratories.

For products as butter biscuits or chocolate bars, total fat content repeatability (r) and reproducibility (R):

content range 1.5-50% (m/m): r = 0.7% (m/m) R = 1.5% (m/m)