18. Determination of total fat content in natural products and foodstuffs – the extraction method

Lipids constitute very big and differential, with regard to their chemical character, group of natural organic compounds which a common feature is lipophilicity [1]. It means that affinity to this group is defined not by the chemical composition (presence of the same functional groups like for peptides, proteins or sugars) but solubility, i.e. ability to extract them from biological materials by extraction with nonpolar solvents, like ethers, chloroform, toluene or petroleum.

Following organic compounds include to the lipids:
- fats – esters of glycerol and fatty acids,
- waxes – esters of fatty acids and long chain alcohols,
- phospholipids, cerebrosides and sulfolipids – they contain, besides residues of fatty acids and alcohols, residues of other compounds like aminoacids, esters of phosphoric acid or carbohydrates,
- lipids derivatives – fatty acids, steroids, long chain alcohols and others organic compounds.

Lipids, beside proteins and carbohydrates, are fundamental constituents of living organisms. They are also fundamental ingredients of nourishments, fulfill an energetic functions and also are substrates in biosynthesis of other important substances.

Edible fats are categorized by origin to vegetable, animal and modified fats, by state of matter to oils and solid fats. Fats are produced by pressing, spinning, smelting, extraction or combination of above processes. Fats extracted from organic materials are called raw fats. It should be noticed that extracted raw fats contain also others non-fatty lipophilic substances.

Precise quantitative analysis of lipids in foodstuffs [2] is important not only for nutritional labeling [3, 4], but also for determining whether the food meets the standards for identity and uniformity, and for understanding the effects of fats and oils on the functional and nutritional properties of foods. The validity of the fat analysis of a food depends on many factors, including proper sampling and preservation of the sample before the analysis [5].

Because of commercial regulations, it is important for foods producers to be able to report fat content in a serving size of food item [4]. International standards of fats and oils analysis usually base on extraction methods [6-17], including Soxhlet method.

Extraction. Extraction means separation of substance from one phase, in which it is dissolved or suspended, to another liquid phase [18]. This process is possible, because substance is distributed between two phases in certain proportions. It is defined by Nernst's distribution law: $K = c_A / c_B$.

According to this law proportion of concentrations c of substance dissolved in non-miscible and being in equilibrium two liquid phases A and B at given temperature is constant (distribution constant K). In this form Nernst's distribution law is applicable only to very diluted solutions (ideal conditions) and to solute, which is identically associated in both phases.

Extraction is only efficient when substance dissolves much more better in one of two phases, i.e. value of distribution constant significantly exceeds one.

For substance described by $K < 100$ (when c_A, the concentration in K definition in extracting phase) simple extraction is not efficient but only multiple extraction with use of new portion of solvent.

In the ideal case two substances (described by constants K_1 and K_2) could be separated independently between to phases. If the difference between the distribution constants of these substances is sufficiently large, they can be separated with use of simple extraction. Difficulty level of distribution is defined by partition coefficient $\beta = \dfrac{K_1}{K_2}$. Two substances could be efficiently separated by extraction only when $\beta > 100$. Separation of substances described by $\beta < 100$ is possible only with multiple extraction.

Extraction of solids with Soxhlet method. The Soxhlet method [19] is one of the most popular methods used for lipid extraction from the natural products and foodstuffs. According to this method lipids are extracted from the solids by multiple rinsing out with an organic solvent like hexane or low-boiling petroleum ethers. Analyzed sample is ground into small particles, dried and placed in a porous cellulose thimble. The thimble is placed in an extractor chamber (2), which is suspended above a distillation flask containing an organic solvent (1) and below a condenser (4). The flask is heated and the solvent partially evaporates and moves up into the condenser where it is converted into a liquid that trickles down into the extraction chamber containing the sample. When the solvent surrounding the sample exceeds an upper level of an extractor siphon it overflows and trickles back down into the distillation flask. At the end of the multiple extraction process, which usually takes several hours, the extractor is removed and the organic solvent is distilled out from the extracted lipid. Some devices [20] are provided with a funnel (3), which allows to recover the organic solvent at the and of the extraction process after closing a stopcock between the funnel and the extraction chamber without earlier dismantling of apparatus. When the whole organic solvent is evaporated, the flask with the remaining liquid is weighed and the mass of the extract (total fat) is measured. The percentage of lipid in the initial sample can then be calculated.

Aim of exercise The aim of this activity is the extraction of the chosen sample, like French fries, potato chips, sunflower seeds, rape seeds, pumpkin seeds, animal feeding stuffs, oilseeds meals and others with *n*-hexane in Soxhlet apparatus and gravimetric determination of total fat in relation to the mass of the analyzed sample.

EXPERIMENTAL SECTION

Caution. During analysis highly flammable and volatile organic solvents are used. Safety precautions should be taken. Avoid sources of an open fire. Solvents must be disposed of in appropriate waste containers.

Extraction, distillation and post-extraction evaporation steps must be performed in a vented hood!

Materials	Chemicals
- Soxhlet extractor, - Allihn condenser, - rubber tubing, - round-bottomed flask with ground glass joint per 1000 ml, - glass bottle with ground glass joint per 500 cm^3, - heating mantle, - laboratory stand, - condenser clamp + clamps holder, - cellulose thimble, - fat-free cellulose wool, - glass beads, - mortar and pestle, - crystallizer, - analytical balance - laboratory spoons, - round-bottom flask support, - hot-hand protector.	- *n*-hexane $CH_3(CH_2)_4CH_3$, - anhydrous sodium sulfate Na_2SO_4 dried at 150 °C, - nitrogen gas N_2.

PROCEDURE

Sample preparation. Place an analyzed sample into a mortar and grind it with a pestle into a homogeneous mass.

Weigh about 40 g (±1 mg) of prepared sample in crystallizer on analytical balance, m_0 (write down in a lab-book), and next mix the sample with about 10 g of anhydrous sodium sulfate. Transfer the mixed sample quantitatively to the cellulose thimble (remains of sample should be wipe off with the cellulose wool and transfer into the thimble). Plug the thimble with a fat-free cellulose wool. The thimble should be filled maximally to the three-fourths full.

Analysis. Add 3-5 glass beads into the clean and dry extraction flask and weigh it on the analytical balance with ±1 mg accuracy. Write down the result in the lab-book (m_1). Place the flask in the heating mantle and pour carefully about 350 ml of *n*-hexane. Join the Soxhlet extractor with the flask. Place the thimble with the sample into the chamber of the extractor. Join the condenser with the extractor. Join the rubber tubing with the condenser – cooling water should enter through the lower fitting and exit through the upper fitting. Ask the instructor to check the correctness of the connections of apparatus.

Turn on the cooling water flow and begin heating the flask with a mantle. Extract the sample for approximately 2 – 2,5 hours to collect the extract in *n*-hexane. Adjust heat as necessary to achieve about 10 flushes per hour.

After completed extraction (about 40 minutes before the end of the lab session) open a stopcock at the bottom of the extraction chamber and distill out the whole solvent from the extracted lipid to the glass bottle with ground glass joint. Disconnect the electric power, turn off the water and cool down the apparatus. Disconnect the condenser and Soxhlet extractor, and place the extraction flask on a round-bottom flask support using a hot-hand protector. Remove the remains of the solvent by flowing a steady stream of nitrogen gas over the sample for about 10 minutes. Cool down the flask and weigh it on the analytical balance with ±1 mg accuracy. Write down the result in the lab-book (m_2).

Calculations. Total fat content, *H*, expressed in grams per 100 g of sample (or in percentage) could be calculated from the following formulae:

$$H = \frac{m_2 - m_1}{m_0} \cdot 100 \qquad \left[\frac{g}{100g} \quad \text{or} \quad \% \right]$$

where:

m_0 – is the initial mass of the analyzed sample,

m_1 – is the mass of the clean extraction flask containing the glass beads,

m_2 – is the mass of the extraction flask containing the extracted lipid and the glass beads after solvent evaporation and cooled down to room temperature.

All the masses are expressed in grams [g].

Report

The report should be composed of:

> ➢ first name and last name of the person conducting the analysis,
> ➢ analysis' date,
> ➢ all the necessary information enable to sample identification,
> ➢ short description of sample preparation and analysis,
> ➢ analysis' result (if possible, comparison of the analysis' result and the total fat content provided by the producer from the product label),
> ➢ comment.

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