INSTITUTE OF CHEMICAL TECHNOLOGY, PRAGUE
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ANALYSIS OF FOOD AND NATURAL PRODUCTS – LABORATORY EXERCISE

Determination of fat and its quality evaluation

(Soxhlet method for fat extraction and titrimetric determination of acid value)

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Required knowledge

1. Lipid chemistry: lipid definition, structure and classification, chemical composition of fat, fat content in different foods, lipid reactions occurring during food storage and processing.
2. Titrimetric methods: principle, basic definitions and rules, related calculations.
3. Safety and fire protection rules.

Control questions

- What types of chemical compounds are present in olive oil?
- What are the main differences between olive oil, coconut fat and pork lard?
- What is the fat content in the following food commodities?
  - milk
  - fried chips
  - Coca-cola
  - butter
- What types of chemical compounds are present in olive oil after its using for frying?

- How do you prepare 0.1 mol/l potassium hydroxide solution (one litre)? How correctly must you work? Why? (This solution will be used for titrimetric determination of acids.)

- Which safety rules are important during work with
  - KOH,
  - diethyl ether,
  - methanolem?

Laboratory exercise content

1. **Fat determination using Soxhlet extraction**
   (according to EN ISO 659; adapted for students laboratory course and Soxtec apparatus)

   Tasks and questions:
   - Determine fat content in one sample of nuts. Realize six parallel determinations.
   - Express your results as the confidence interval.
   - What is the petroleum ether?
   - How can you determine fat content in milk and in meat?

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2. **Acid value titrimetric determination**
   (according to Commission Regulation (EEC) No. 2568/91)

   Tasks and questions:
   - Determine (at least two times) exact concentration of potassium hydroxide solution using oxalic acid (dihydrate).
   - Do blank test at least two times.
   - Analyze your sample at least three times.
   - Express the results as acid value and as acidity.
   - Does your sample correspond to European legislation?

Fat determination

Scope and field of application

This international standard specifies a reference method for the determination of the hexane extract (or petroleum ether extract), called "oil content", of oil seeds used as industrial raw materials (i.e. rapeseed, soya beans, sunflower and other seeds used for plant oils production). However, the identical method can be also used for fat determination in other food materials with high fat content and lower water content (i.e. biscuits, fried chips, nuts, etc.).

Principle

Extraction of a test portion, in a suitable apparatus, with technical hexane or petroleum ether. Elimination of the solvent and weighing of the extract obtained.

Apparatus

- extraction unit 1043, Soxtec System HT6
- service unit 1046, Soxtec System HT6
- extraction cups (1)
- cup holder (2)
- tongs for extraction cups
- thimbles (3)
- thimble adapters (4)
- thimble stand (5)
- thimble support (6)
- thimble handler (7)
- holder of thimble support (8)

1 

2 

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6 

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Reagents

- petroleum ether

Procedure

- Turn on the Service Unit and start to heat up the oil bath (adjusted temperature will be 110 ºC).
- Open the cold water tap for the reflux condensers.
- Attach the thimbles to the adapters.
- Weigh the sample into the thimbles (use the thimble support).
- Move the thimbles to the thimble stand (use the thimble handler).
- Put a cotton plug on the top of the sample and place the thimbles into thimble support attached to the holder (use again the thimble handler).
- Insert the thimbles into the Extraction Unit.
- Weigh the extraction cups (with boiling chips).
- Insert the extraction cups, each with 50 ml of extraction solvent, into the Extraction Unit (use the cup holder).
- Move the extraction mode knobs to the “BOILING” position (thimbles will now be immersed in the solvent) and extract your samples 25 minutes. Make sure that the condenser values are open.
- Move the extraction mode knobs to the “RINSING” position (thimbles will now hang above the solvent surface) and continue in extraction 20 minutes.
- Close the condenser valves and remove extraction solvent from the extracts (after 15 minutes, press the “AIR” button on the Service Unit and open the “EVAPORATION” valve on the Extraction Unit for 5 minutes).
- Release the extraction cups from the Extraction Unit.
- Dry the extracts (the extraction cups) in an oven with adjusted temperature between 103 and 105 ºC for 20 minutes.
- Cool the extraction cups in a desiccator (20 minutes) and weigh them.

Acid value determination

Scope

This standard describes method for the determination of the free fatty acid content of animal and vegetable oils and fats. This content can be expressed as an acid value, or as acidity.

Field of application

This method is applicable to animal and vegetable oils and fats. It is not applicable to waxes.

Definition

- The acid value is the number of mg of potassium hydroxide required to neutralize the free fatty acids in 1 g of the fat.
- The acidity is an expression of the content (in %, m/m) of free fatty acids as content of dominant or chosen fatty acid. According to the nature of the fat it can be expressed as in Table 1.

Table 1

Nature of fat	Expressed as	Molecular weight
Coconut, palm kernel and similar oils	Lauric acid	200
Palm oil	Palmitic acid	256
All other oils	Oleic acid	282

When the result is reported as acidity, but not further defined, then conventionally the acidity is always expressed as oleic acid.

Principle

Solution of a known quantity of the fat to be analysed in a mixture of ethanol and diethyl ether, followed by titration of the free fatty acids present with an ethanolic solution of potassium hydroxide.

Apparatus

- burette
- titration vessels

Reagents

- solvent mixture 1/1 (V/V) of 95 per cent (V/V) ethanol and diethyl ether
- potassium hydroxide, about 0.1 mol/l solution in ethanol
  (the exact concentration should be known)
- phenolphthalein, 10 g/l solution in 95 per cent (V/V) ethanol

Procedure

- Determine size of sample according to the Table 2.

Table 2

Expected acid value	Mass of test portion, in g	Weighing accuracy, in g
< 1	20	0.05
1-4	10	0.02
4-15	2.5	0.01
15-75	0.5	0.001
>75	0.1	0.0002

- Weigh the test portion into a titration vessel.
- Dissolve it in 50 ml of the solvent mixture of ethanol and diethyl ether.
- Add 0.2 ml of phenolphthalein solution and titrate, with shaking, with the solution of potassium hydroxide in ethanol to the pink colour persisting for at least 10 seconds.
- Carry out simultaneously a blank test.

Appendix

- COMMISSION REGULATION (EEC) No. 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis (amended by consecutive regulations)