INSTITUTE OF CHEMICAL TECHNOLOGY, PRAGUE
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Department of Food Analysis and Nutrition

ANALYSIS OF FOOD AND NATURAL PRODUCTS - LABORATORY EXERCISE

Determination of fatty acids
(method: Gas chromatography with flame ionization detector)

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CONTENT

Required knowledge

- the principle of the method and its applicability
- the principle of gas chromatography; commonly used detectors
- evaluation of chromatographic results using the internal normalization method

Evaluative critera

- proved theoretical knowledge
- quality of practical work execution
- compliance with laboratory work rules (safety, order)
- protocol – integrity of experimental data, results calculation and discussion

Laboratory exercise content

1. Preparation of methyl esters of fatty acids ČSN EN ISO 5509 (adapted for teaching)
2. Analysis of fatty acid methyl esters by gas chromatography; ČSN ISO 5508

specification of the exercise:

- Each student performs one determination, one pair of students analyses the same sample.
- Based on the comparison with standard chromatograms of the fatty acid profiles in vegetable oils (available from teacher assistant) the students determine the type of analysed oil.

specification - filling talks:

basic approach in determining the profile of fatty acids
influence of the method used, the possibility of using an internal standard
factors influencing the choice of methods for the determination of fatty acids:

- the chain length and configuration of fatty acids
- requirements for determination - determination of the single-flower oil x determination of certain fatty acids

Operating procedure for the determination of fatty acid methyl esters

1. SCOPE

The method is applicable to the determination of fatty acid methylesters (FAMEs) in vegetable oils. The method is unsuitable for the determination of fatty acids with a high number of epoxy-, hydroperoxy-, cyclopropyl-, cyclopropenyl-, ketone- and aldehyde groups, or for fatty acids containing less than six C atoms.

2. BASIC PRINCIPLE

The method is based on the saponification of glycerides and phospholipids. Esterification of free fatty acids in alkaline methanol is then carried out. After extraction by heptane fatty acid, methylesters are analysed using gas chromatography with flame-ionization detection. The content of individual fatty acids is expressed as a percentage of the total content of all acids in the sample.

3. EQUIPMENT

- Gas chromatograph, Hewlett-Packard HP 4890A equipped with a flame-ionisation detector
- fused silica capillary column HP-INNOWAX (30 m x 0.25 mm x 0.25 μm), (stationary phase polyethylenglycol)
- nitrogen (99.998 %)
- hydrogen (99.9 %)
- air (99.9 %)
- glass syringe 10 μL - Hamilton (USA)
- analytical balance
- heater
- electric hot plate
- reflux condenser according to Dimroth
- common laboratory glassware

4. CHEMICALS AND SOLUTIONS

All chemicals of purity p.a.:
- methanol
- sodium hydroxide – 0.5 mol/L solution in methanol
- sodium chloride – saturated solution *(NaCl solubility: 36.0 g in 100 g water)*
- heptane
- distilled water

5. PROCEDURE

5.1 Preparation of saturated NaCl solution

Weigh approximately 160 g of NaCl into a beaker. Use a scuttle to transfer NaCl into a 1000 mL Erlenmayer flask. Add 400 mL of distilled water and heat the flask on an electric hot plate, shaking the content occasionally.

5.2 Preparation of fatty acid methylesters (FAMEs)

Method A:

Weigh 250 mg of sample (to a precision of 4 decimal places) into a 100 mL round-bottomed flask using a Pasteur pipette. Add a boiling stone, and 6 mL of methanolic solution of NaOH (c = 0.5 mol/L). Attach the flask to a side-arm Y-piece and reflux condenser, and heat the content. Add 10 mL of heptane using the side-arm of the Y-piece, and heat for one more minute. Switch the heater off, add saturated solution of NaCl and shake in a circular fashion. Fill the flask with saturated solution of NaCl such that the heptane layer reaches the narrow part of the flask. Let the flask cool down, and the phases separate. Using a Pasteur pipette transfer 1 mL of the upper (heptane) layer into a GC vial.

Warning: *Use a pipette filler to handle methanolic NaOH solution and heptane. Use a beaker to add saturated solution of NaCl.*

Warning: ***Only switch the heater on*** *(heating under a reflux condenser)* ***IN THE PRESENCE OF INSTRUCTOR,*** *and only when you have all the chemicals and glassware ready to hand**!!***

Method B: A screening method - cold transesterification

Weigh 0.1 g (to a precision of 2 decimal places) of sample (pure fat or oil) into a 15 ml cuvette, add 5 ml of isooctane and dissolve the fat. Add 0.5 ml of 2 M methanolic KOH solution and shake for 8 minutes. Then leave the cuvette at room temperature for 6 minutes. Using a Pasteur pipette transfer 1 mL of the upper (isooctane) layer into a GC vial.

5.3 Gas chromatographic analysis of FAMEs

5.3.1 GC conditions

Gas chromatograph: Hewlett-Packard HP 4890A coupled with flame-ionization detector

Column: HP-INNOWAX (30m x 0.25mm x 0.25μm)

Carrier gas: nitrogen, flow 0.5 mL /min at 150 ^{o}C
Injection: 1 μL, split 25:1
Injector temperature: 250 ^{o}C
Detector temperature: 300 ^{o}C
Column temperature programme **A**: 150 ^{o}C (1 min), 5 ^{o}C/min to 230 ^{o}C (5 min),
15 ^{o}C/min to 245 ^{o}C (8 min), Run time 31 min

Column temperature programme **B**: 150 ^{o}C (1 min), 50 ^{o}C/min to 200 ^{o}C (5 min),
10 ^{o}C/min to 250 ^{o}C (6 min), Run time 18 min

5.3.2 Identification and quantification

Identification of individual fatty acids is based on a comparison of the retention times and chromatographic profiles measured in the sample with the retention times and profiles shown in the enclosed chromatograms of standard oils.

The content of individual fatty acids is expressed as a percentage of the total content of all acids in the sample.

6. INTERPRETATION OF RESULTS

Oil type is determined by a comparison of the content (as a percentage) of individual fatty acids (mainly palmitic, stearic, oleic, linolic and linoleic acid) in the sample and in standard oils.

The decision whether or not the sample meets the requirements for fatty acid content in a particular type of oil is based on a comparison of the content in the sample with the content´s range as specified in legislation for each type of oil (Regulation 90/2000 Sb.)

Appendix: Chromatograms and fatty acid content (%) in standard soy, sunflower and olive oil.