

STANDARD OPERATION PROCEDURE
Faculty of Biosciences, NMBU

Method name: Fatty acid composition (FAME)

BIOVIT No. : Arb1046

1. Introduction

Being able to analyze fatty acids / fatty acid composition has become more and more important due to an increased awareness of their nutritional and health effects (1).

Long-chain fatty acids exist naturally as tri-esters with glycerol in oils, fats and waxes from plants and animals. Gas chromatography (GC) is the most suitable method for routine analysis of fatty acids, but first the fatty acids must be released from the glycerol unit (by saponification) and then derivatized to volatile fatty acid methyl esters (FAME), before they can be extracted into heptane.

With this method (1), the fatty acids are synthesized and extracted directly from various crude samples and dry samples, without the need to perform an organic solvent extraction first. The results are stated both as relative area percentages (fatty acid profile) and quantitatively in g/kg.

The method is standardized for approx. 40 μ L grease / oil.

2. Reagents

- Heptane: HPLC grade
- 10 N KOH: 561.1 g/L water
- MeOH: HPLC grade
- 24 N H₂SO₄: 663 mL concentrated acid per L water
- Internal standard of choice: 0.5mg/mL - C13: 0, C19: 0, C23: 0

3. Risk assessment

Heptane: Harmful, very flammable, harmful to the environment.
Wear suitable gloves, work in a fume hood.

KOH: Harmful if swallowed, very corrosive.
Wear gloves, goggles

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Methanol: Highly flammable, toxic.
Risk of serious damage to health by inhalation/in contact with skin/if swallowed.

H₂SO₄: Reacts violently with water. Highly corrosive, irritates the respiratory tract.
Water must not be added. (always add acid to water)
Rinse immediately with plenty of water if you get it in your eyes. Contact a doctor. Wear suitable protective equipment. See data sheet.

4. Equipment

- Water bath
- Test tubes with tight screw caps 10 - 12 mL
- Vortex mixer
- Swing- out centrifuge
- Trace GC Ultra with auto injector

5. Sample material

Grass, concentrates, feed, milk, meat/tissue and oils.

Crude samples can be fresh or frozen and about 0.8 g is needed. For pure oils, weigh in less (<0.05 mL). For dry samples, weigh in about 0.3 g.

6. Special remarks

The method can also be used on smaller sample volumes - see details in the next section

7. Job description

The method has been modified. The volumes are scaled down compared to the reference (1).

- 1) The sample is weighed directly into test tubes, 0.3 g dry sample or 0.8 g crude sample
- 2) Add 4.25 mL of MeOH
(If internal standard is not to be added, increase the volume of methanol accordingly)
- 3) Add internal standard: 0.5 mL
(normally C13: 0, alternatively: C19: 0 or C23: 0) - concentration: 0.5 mg / mL)
- 4) Add 0.56 mL of 10 N KOH
- 5) Put the screwcaps on the tubes and shake vigorously for 1min on vortex mixer

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- 6) Place the tubes in a water bath at 55 °C for 1.5 hours
- 7) During the incubation period, the tubes should be shaken vigorously 5 times, approx. every 20 min
- 8) Cool in running water (or ice bath)
- 9) Add 0.465 mL of 24N H₂SO₄
- 10) Place the tubes in a water bath at 55 °C for 1.5 hours
- 11) During the incubation period, the tubes should be shaken vigorously 5 times
- 12) Cool in running water (or ice bath)
- 13) Add 2.4 mL of Heptane
- 14) Shake vigorously for at least 1.5 minutes on a vortex mixer
- 15) Centrifuge the tubes in Swing-out centrifuge: 5 min at 3000 rpm, (room temperature)
- 16) About 1.5 mL of the heptane layer is transferred to 2 mL GC vials with lid.

Small samples

The analysis can be performed on very small samples: liver, intestine, muscle from fish (0.05 - 0.2g) The samples are extracted and methylated as usual, but with reduced volumes:

- 0.1 mL internal standard
- 0.28 mL 10 N KOH
- 2.1 mL of MeOH
- 0.232 mL 24 N H₂SO₄
- 1.2 mL of heptane

The heptane layer is transferred to 3 mL glass tubes, evaporated to dryness under nitrogen and redissolved in 0.2 mL of heptane. Transfer to 0.3 mL GC vials with lid.

Analysis of the fatty acid composition on GC-FID

- GC system: Trace GC Ultra with auto injector (Thermo Scientific)
- Software: Chromeleon 7.2 (Thermo Scientific)
- Column: Rt - 2560, 100 m, 0.25 mm ID, 0.20 µm dt (Restek, Cat # 13198)
- Injector temp: 250 °C

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- Split injection: 1:40 split ratio
- Injection volume: 1 µL
- Carrier gas: Helium
- Constant pressure: 2.70 bar
- Oven temp: 140 °C (5 min) to 240 °C at 4 °C / min
- Detector: FID; temp. 250 °C
- Analysis time: 50 min
- Instrument Method: FAME_Trace
- Processing method: supelco37_jan 2016

To create a sequence in Chromeleon see “Arb 1050 amino acid analysis”.

Start sequence with blank (heptane) and standard (Supelco37 comp.Mix - located at -20 °C).

Include a standard per 10th sample. If a large number of samples are to be analyzed, precision and sensitivity should be checked along the way + make sure that washing solutions do not run out. If the sensitivity drops a lot, the column must be baked out at 200 °C overnight. If there are a lot of "spikes" in the chromatogram, the detector must be restarted.

8. Calculation

Chromeleon gives the results in the relative area%.

To calculate the results in g/kg enter «relative area%» in excel sheet: Calculation FAME (*labmal-diverse analyser-FAME*)

$$\frac{\text{Amount of internal standard (mg)} \times \text{Relative area\% fatty acid}}{\text{Relative area\% internal standard}} = \text{Amount of fatty acid (mg)}$$

$$\frac{\text{Amount of fatty acid (mg)}}{\text{Weighed quantity of sample (g)}} = \text{g/kg}$$

9. Reference:

O'fallon, J.V., 2007. A direct method for fatty acid methyl ester synthesis: Application to wet meet tissues, oils and feedstuff. *Journal of Animal Science*, 85: 1511-1521

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