INTERNATIONAL STANDARD

ISO 6800

Second edition
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Animal and vegetable fats and oils — Determination of the composition of fatty acids in the 2-position of the triglyceride molecules

Corps gras d'origines animale et végétale — Détermination de la composition des acides gras en position 2 dans les triglycérides

Reference number
ISO 6800:1997(E)

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 6800 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 11, *Animal and vegetable fats and oils.*

This second edition cancels and replaces the first edition (ISO 6800:1985), which has been technically revised.

Annexes A to C of this International Standard are for information only.

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Animal and vegetable fats and oils — Determination of the composition of fatty acids in the 2-position of the triglyceride molecules

1 Scope

This International Standard specifies a method for the determination of the composition of fatty acids which are esterified in the 2-position (*ß* or internal position) of the triglyceride molecules in animal and vegetable fats and oils.

Because of the nature of pancreatic lipase action, the method is applicable only to fats and oils with a melting point below 45 °C.

The method is not unreservedly applicable to all fats and oils, particularly those containing substantial amounts of

- fatty acids with 12 or fewer carbon atoms (e.g. copra oil, palm kernel oil, butyric butter fats);
- fatty acids with 20 and more carbon atoms and of a high degree of unsaturation (more than four double bonds) (e.g. fish oil and marine animal oil);
- fatty acids which have secondary groups containing oxygen.

NOTE — Fatty acids with double bonds in the (*n*-16) to (*n*-11) position (e.g. petroselinic acid) are converted only very slowly by pancreatic lipase. This may lead to wrong results.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 660:1996, *Animal and vegetable fats and oils – Determination of acid value and of acidity.*

ISO 661:1989, *Animal and vegetable fats and oils – Preparation of test sample.*

ISO 3696:1987, *Water for analytical laboratory use – Specification and test methods.*

ISO 5508:1990, *Animal and vegetable fats and oils – Analysis by gas chromatography of methyl esters of fatty acids.*

ISO 5509:—[1] *Animal and vegetable fats and oils – Preparation of methyl esters of fatty acids.*

3 Principle

After neutralization, where necessary, of any free fatty acids, purification of the test portion by column chromatography. Partial enzymatic hydrolysis of the glycerides to yield 2-monoglycerides. Separation of the monoglycerides by thin-layer chromatography (TLC) and determination of their fatty acid composition by gas chromatography.

4 Reagents

Use only reagents of recognized analytical grade and water in accordance with grade 2 of ISO 3696.

4.1 Reagents for the purification of the test portion

4.1.1 2-Propanol, or **ethanol**, 95 % (*V/V*).

4.1.2 Hexane (if available) or **light petroleum** (boiling range 30 °C to 60 °C).

4.1.3 2-Propanol, 50 % (*V/V*), or **ethanol**, 50 % (*V/V*).

4.1.4 Sodium hydroxide, 0,5 mol/l solution.

4.1.5 Phenolphthalein solution, 1 g per 100 ml of ethanol, 95 % (*V/V*).

4.1.6 Activated neutral alumina, for chromatography, Brockmann activity I, recently activated for 2 h at 260 °C and kept in a desiccator.

4.1.7 Nitrogen.

4.2 Reagents for hydrolysis of the triglycerides

4.2.1 Diethyl ether, free from peroxides.

4.2.2. Hydrochloric acid, 6 mol/l solution.

4.2.3 Sodium cholate, 1 g/l solution of enzymatic quality.

4.2.4 Calcium chloride, 220 g/l solution.

4.2.5 Buffer solution, 2-amino-2-(hydroxymethyl)propan-1,3-diol[2], 1 mol/l, adjusted to pH 8 with hydrochloric acid (4.2.2) using a pH-meter.

Store this solution at between 0 °C and 4 °C and use within 14 days.

1) To be published. (Revision of ISO 5509:1978)
2) Alternative names are: tris(hydroxymethyl)methylamine; tris(hydroxymethyl)aminomethane.

4.2.6 Pancreatic lipase, with an activity of between 8 and 20 units/mg.

Store dry in a refrigerator. Before use, bring a portion of the powder to ambient temperature.

NOTE — Lipase of suitable activity is available commercially. If preferred, the lipase may be prepared and assayed in accordance with the procedure described in annex A.

4.3 Reagents for the isolation of the 2-monoglycerides

4.3.1 Ethanol, 95 % (*V*/*V*).

4.3.2 Hexane (if available) or **light petroleum**, boiling range 30 °C to 60 °C.

4.3.3 Acetone.

4.3.4 Silica powder, with binder, for thin-layer chromatography.

4.3.5 Developing solvent, prepared as follows:

hexane (if available) or light petroleum:	70 ml
diethyl ether:	30 ml
formic acid, 98 % (*V*/*V*) min.:	1 ml

4.3.6 2',7'-Dichlorofluorescein, indicator solution, 2 g/l in ethanol, rendered slightly alkaline by addition of a drop of 1 mol/l sodium hydroxide per 100 ml of the solution.

4.4 Reagents for the analysis of the 2-monoglycerides by gas chromatography

See ISO 5508 and ISO 5509.

5 Apparatus

Usual laboratoy equipment and, in particular, the following.

5.1 Apparatus for the purification of the test portion

5.1.1 Water bath, thermostatically controlled, and capable of being maintained at 30 °C to 40 °C.

5.1.2 Glass column, for chromatography, 13 mm internal diameter and 400 mm in length, equipped with a sintered glass plate and a tap.

5.1.3 Rotary evaporator, with 250 ml flask.

5.1.4 Tubing, for bubbling nitrogen.

5.1.5 Separating funnel, of 500 ml capacity.

5.1.6 Round-bottom flask, of 100 ml capacity.

5.2 Apparatus for the hydrolysis of the triglycerides

5.2.1 Centrifuge.

5.2.2 Centrifuge tube, glass, of 10 ml capacity, with ground stopper.

5.2.3 Vibrating electric shaker, for vigorous agitation of the centrifuge tube.

5.2.4 Water bath, thermostatically controlled, and capable of being maintained at 40 °C ± 0,5 °C.

5.2.5 Hypodermic syringe, of 1 ml capacity, with thin needle.

5.2.6 Stopwatch.

5.3 Apparatus for the isolation of the 2-monoglycerides

5.3.1 Developing tank, for thin-layer chromatography, with ground glass lid, suitable for containing glass plates 200 mm x 200 mm.

5.3.2 Spreader and rack, for preparation of the plates.

5.3.3 Glass plates, 200 mm x 200 mm.

5.3.4 Microsyringe, capable of dispensing drops of 3 µl to 4 µl.

5.3.5 Apparatus for spraying the indicator solution onto the plates

5.3.6 Microspatula.

5.3.7 Oven, capable of being maintained at 103 °C ± 2 °C.

5.3.8 Ultraviolet lamp, for examining chromatographic plates, for example with a wavelength of 254 nm.

5.3.9 Round-bottom flask, of 25 ml capacity, with **air condenser** of approximately 1 m length, with ground joint.

5.3.10 Conical flask, of 250 ml capacity, with ground stopper.

5.3.11 Conical flask, of 50 ml capacity (if necessary).

5.3.12 Filter, of sintered glass, porosity P 40 (16 µm to 40 µm) (if necessary).

5.3.13 Desiccator, containing an efficient desiccant.

5.4 Apparatus for the analysis of the 2-monoglycerides by gas chromatography

See ISO 5508 and ISO 5509.

6 Sampling

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport and storage.