SLOVENSKI STANDARD

SIST ISO 5509

prva izdaja
junij 1995

Rastlinske in živalske maščobe in olja - Priprava metil estrov maščobnih kislin (prevzet standard ISO 5509:1978 z metodo platnice)

Animal and vegetable fats and oils - Preparation of methyl esters of fatty acids

Corps gras d'origines animale et végétale - Préparation des esters méthyliques d'acides gras

Deskriptorji: maščobe, živalske maščobe, rastlinske maščobe, rastlinska olja, maščobne kisline, metil ester, preskusni vzorci

ICS 67.200.10

Referenčna številka
SIST ISO 5509:1995 (en)

Nadaljevanje na straneh od II do III in 1 do 6

UVOD

Standard SIST ISO 5509, Rastlinske in živalske maščobe in olja - Priprava metil estrov maščobnih kislin, prva izdaja, 1995, ima status slovenskega standarda in je z metodo platnice prevzet mednarodni standard ISO 5509, Animal and vegetable fats and oils - Preparation of methyl esters of fatty acids, first edition, 1978-10-15.

PREDGOVOR

Mednarodni standard ISO 5509:1978 je pripravil tehnični odbor Mednarodne organizacije za standardizacijo ISO/TC 34 Kmetijski pridelki in živilski proizvodi.

Odločitev za prevzem tega standarda po metodi platnice je sprejela delovna skupina WG 2 Oljnice ter rastlinske in živalske maščobe in olja v okviru tehničnega odbora USM/TC Kmetijski pridelki in živilski proizvodi.

Ta slovenski standard je dne 1995-06-16 odobril direktor USM.

ZVEZA S STANDARDI

Ta standard skupaj z naslednjimi slovenskimi standardi, prevzetimi mednarodnimi standardi ISO, ureja kontrolo kakovosti oljnic ter rastlinskih in živalskih maščob in olj:

SIST ISO 542 (en)	Oljnice - Vzorčenje
SIST ISO 658 (en)	Oljnice - Določanje vsebnosti nečistoč
SIST ISO 659 (en)	Oljnice - Določanje heksanskega (ali petroleterskega) ekstrakta, imenovanega "vsebnost olja"
SIST ISO 661 (en)	Rastlinske in živalske maščobe in olja - Priprava preskusnega vzorca
SIST ISO 664 (en)	Oljnice - Zmanjšanje laboratorijskega vzorca na preskusni vzorec
SIST ISO 665 (en)	Oljnice - Določanje vsebnosti vlage in hlapnih snovi
SIST ISO 729 (en)	Oljnice - Določanje kislosti olja
SIST ISO 5508 (en)	Rastlinske in živalske maščobe in olja - Določanje sestave maščobnih kislin z metodo plinske kromatografije
SIST ISO 5555 (en)	Rastlinske in živalske maščobe in olja - Vzorčenje

OSNOVA ZA IZDAJO STANDARDA

- Prevzem standarda ISO 5509:1978.
- Ta slovenski standard pokriva področje JUS E.K8.038:90.

OPOMBI

- Povsod, kjer se v besedilu standarda uporablja izraz "mednarodni standard", to pomeni v SIST ISO 5509:1995 "slovenski standard".
- Uvod in predgovor nista sestavni del standarda.

INTERNATIONAL STANDARD 5509

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION•МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ•ORGANISATION INTERNATIONALE DE NORMALISATION

Animal and vegetable fats and oils — Preparation of methyl esters of fatty acids

Corps gras d'origines animale et végétale — Préparation des esters méthyliques d'acides gras

First edition — 1978-10-15
Corrected and reprinted —

UDC 664.3 : 661.73 **Ref. No. ISO 5509-1978 (E)**

Descriptors : fats, animal fats, vegetable fats, vegetable oils, fatty acids, methyl ester, test specimens.

Price based on 6 pages

FOREWORD

ISO (the International Organization for Standardization) is a worldwide federation of national standards institutes (ISO member bodies). The work of developing International Standards is carried out through ISO technical committees. Every member body interested in a subject for which a technical committee has been set up 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.

Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council.

International Standard ISO 5509 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in July 1976.

It has been approved by the member bodies of the following countries :

Australia	Hungary	Poland
Austria	Iran	Romania
Canada	Israel	South Africa, Rep. of
Chile	Korea, Rep. of	Spain
Czechoslovakia	Mexico	Thailand
Ethiopia	Netherlands	Turkey
France	New Zealand	United Kingdom
Germany, F.R.	Peru	Yugoslavia

No member body expressed disapproval of the document.

Printed in Switzerland

Animal and vegetable fats and oils — Preparation of methyl esters of fatty acids

1 SCOPE

This International Standard specifies methods of preparing the methyl esters of fatty acids.

The methyl esters so produced can be used in the various analytical procedures requiring such derivatives, for example gas-liquid chromatography, thin-layer chromatography, infra-red spectrophotometry, etc.

2 FIELD OF APPLICATION

2.1 The methods specified in clauses 4 and 5 are applicable to the preparation of methyl esters of fatty acids with 6 or more carbon atoms, from all types of animal and vegetable fats, oils and fatty acids. In the presence of fatty acids with 6 or 8 carbon atoms, and in the case of preparing methyl esters for gas-liquid chromatography, it is essential that the solvent should not be removed from the solution of methyl esters.

The general method using boron trifluoride (clause 4) is to be preferred for most oils and fats, but may lead to erroneous results in the following cases :

— compounds having secondary oxygen groupings (hydroxy-, hydroperoxy-, keto-, epoxy-);

— compounds containing cyclopropane- and cyclopropene- groups;

— conjugated polyunsaturated compounds and acetylenic compounds;

— waxes.

For these it is preferable to use one of the methods described in clause 5. Nevertheless, if the fatty matter contains such compounds only in very small amount (for example cottonseed oil), it may be esterified according to the general method in clause 4.

See also 8.1.

2.2 The special method described in clause 6 is applicable to the preparation of methyl esters of fatty acids with 4 or more carbon atoms, from neutral oils and fats (acid value less than 2), principally for analysis by gas-liquid chromatography.

3 REFERENCE

ISO/R 661, *Crude vegetable oils and fats — Preparation of contract sample for analysis.*

4 GENERAL METHOD USING BORON TRIFLUORIDE

WARNING — Boron trifluoride is poisonous. For this reason, it is not recommended that the analyst prepare the methanolic solution of boron trifluoride from methanol and boron trifluoride (see 8.3).

The methods described involve the use of potentially hazardous reagents. Normal precautions should be taken for eye protection and for protection from the dangers of corrosive chemical burns.

4.1 Principle

Saponification of the glycerides, and esterification of the liberated fatty acids in the presence of boron trifluoride.

4.2 Reagents

Unless stated otherwise, all reagents and solvents shall be of analytical quality, and the water shall be distilled water or water of equivalent quality.

4.2.1 Sodium hydroxide, methanolic solution, approximately 0,5 N.

Dissolve 2 g of sodium hydroxide in 100 ml of methanol containing not more than 0,5 % (*m/m*) of water. If the solution has to be stored for a considerable time, a small amount of white precipitate of sodium carbonate may be formed; this has no effect on the preparation of the methyl esters.

4.2.2 Boron trifluoride, methanolic solution, 12 % to 15 % (*m/m*)[1] (see 8.2).

4.2.3 Heptane, of chromatographic quality (see 8.2 and 8.4).

4.2.4 Light petroleum, redistilled (boiling range 40 to 60 °C), bromine value less than 1, residue-free, or **hexane** (see 8.2).

4.2.5 Sodium sulphate, anhydrous.

4.2.6 Sodium chloride, saturated aqueous solution.

4.2.7 Methyl red, 1 g/l solution in 60 % (*V/V*) ethanol.

4.2.8 Nitrogen, having an oxygen content less than 5 mg/kg.

4.3 Apparatus

Usual laboratory equipment, and in particular :

4.3.1 Flask, 50 ml or 100 ml, with ground neck.

4.3.2 Reflux condenser, 20 to 30 cm effective length, with ground joint to fit the flask (4.3.1).

4.3.3 Boiling aid, fat-free.

4.3.4 Graduated pipette, capacity at least 10 ml and fitted with a rubber bulb; or an **automatic pipette.**

4.3.5 Inlet tube for nitrogen.

4.3.6 Test tube with ground neck and fitted with a ground glass stopper.

4.3.7 Separating funnels, 250 ml.

4.4 Procedure

Because of the toxic character of boron trifluoride, the following operations are best performed under a ventilated hood. It is essential to wash all glassware with water immediately after use.

4.4.1 *Preparation of the test sample*

The test sample shall be dry and clear. Proceed therefore in accordance with ISO/R 661, but heat the sample to just above the melting point.

4.4.2 *Test portion*

Precise weighing is not normally necessary (see 8.8). The size of the test portion is only required in order that the appropriate size of flask (4.3.1) and the quantities of the reagents and solvent may be selected according to the following table :

Test portion	Flask (4.3.1)	NaOH solution (4.2.1)	BF_3 solution (4.2.2)	Heptane (4.2.3)
mg	ml	ml	ml	ml
100 to 250	50	4	5	1 to 3
250 to 500	50	6	7	2 to 5
500 to 750	100	8	9	4 to 8
750 to 1 000	100	10	12	7 to 10

If the methyl esters are intended for an analysis by gas-liquid chromatography, a test portion of about 350 mg is to be preferred (see 8.5). If it is smaller, care should be taken to ensure that the sample is representative.

4.4.3 *Saponification* (see 8.1)

4.4.3.1 General case of fats and oils

Introduce the test portion into the appropriate flask (see 4.4.2). Add the appropriate amount (see 4.4.2) of the methanolic sodium hydroxide solution (4.2.1) and a boiling aid (4.3.3). Fit the condenser (4.3.2) to the flask.

NOTE — In the presence of fatty acids containing more than two double bonds it is recommended that the air in the methanolic solution and in the flask be removed by bubbling nitrogen (4.2.8) through the solution for a few minutes and maintaining a current of nitrogen into the upper part of the condenser during the following saponification.

Boil under reflux until the droplets of fat disappear (this usually takes 5 to 10 min, but in certain exceptional cases it may take longer) (see 8.7). Add the appropriate amount (see 4.4.2) of the methanolic boron trifluoride solution (4.2.2) from the graduated pipette or automatic pipette (4.3.4) through the top of the condenser to the boiling liquid. Proceed in accordance with 4.4.4.

4.4.3.2 Special case of fatty acids

If the sample consists entirely of fatty acids, the saponification step is not necessary.

Introduce the test portion into the appropriate flask (see 4.4.2). Add the appropriate amount (see 4.4.2) of the methanolic boron trifluoride solution (4.2.2) from the

1) 14 and 50 % solutions are available commercially.

graduated pipette or automatic pipette (4.3.4). Fit the condenser (4.3.2) to the flask and bring to the boil.

4.4.4 *Preparation of the methyl esters*

4.4.4.1 Continue boiling for 2 min.

4.4.4.2 Add the appropriate amount (see 4.4.2) of the heptane (4.2.3) (see 8.4) to the boiling mixture through the top of the condenser (the precise amount does not affect the reaction), and continue boiling for 1 min.

Stop heating, cool to room temperature and then remove the condenser. Add a small portion of the saturated sodium chloride solution (4.2.6) and swirl the flask gently several times.

Add more saturated sodium chloride solution to the flask in order to bring the level of liquid into the neck of the flask.

4.4.4.3 Transfer about 1 ml of the upper layer (heptane solution) into a test tube (4.3.6) and add anhydrous sodium sulphate (4.2.5) to remove any traces of water.

This solution will contain about 100 mg/ml of methyl esters and may be injected directly into the column for gas-liquid chromatography (see 7.1).

4.4.4.4 If it is required that the whole of the dry esters should be recovered, transfer the saline solution and the heptane layer to a 250 ml separating funnel (4.3.7). Separate the layers. Retain the heptane solution. Extract the saline solution twice with 50 ml portions of light petroleum or hexane (4.2.4).

Combine the heptane solution and the two extracts, and wash them with 20 ml portions of water until free from acid, using the methyl red solution (4.2.7) as indicator. Dry over anhydrous sodium sulphate (4.2.5), filter and evaporate the solvent on a water bath under a stream of nitrogen (4.2.8) (see 8.6). For test portions less than 500 mg, it is preferable to reduce proportionately the volumes of solvent and water.

5 ALTERNATIVE METHODS NOT INVOLVING THE USE OF BORON TRIFLUORIDE

5.1 Method applicable to neutral fats and oils (acid value less than 2)

5.1.1 *Principle*

Methanolysis of the glycerides in an alkaline medium.

5.1.2 *Reagents*

Unless stated otherwise, all reagents and solvents shall be of analytical quality, and the water shall be distilled water or water of equivalent quality.

5.1.2.1 Methanol, containing not more than 0,5 % (*m/m*) of water.

5.1.2.2 Potassium hydroxide, methanolic solution, approximately 1 N.

Dissolve 5,6 g of potassium hydroxide in 100 ml of methanol (5.1.2.1).

5.1.2.3 Heptane, of chromatographic quality (see 8.2 and 8.4).

5.1.2.4 Sodium sulphate, anhydrous.

5.1.2.5 Nitrogen, having an oxygen content less than 5 mg/kg.

5.1.3 *Apparatus*

Usual laboratory equipment, and in particular :

5.1.3.1 High-speed stirrer and appropriate means of heating (for example a magnetic stirrer equipped with a heater).

5.1.3.2 Flask, 100 ml, with ground neck.

5.1.3.3 Inlet tube for nitrogen.

5.1.3.4 Reflux condenser, with ground joint to fit the flask (5.1.3.2).

5.1.3.5 Boiling aids, fat-free.

5.1.3.6 Separating funnels, 250 ml.

5.1.3.7 Conical flask, 50 ml, with narrow neck.

5.1.4 *Procedure*

5.1.4.1 PREPARATION OF THE TEST SAMPLE

The test sample shall be dry and clear. Proceed therefore in accordance with ISO/R 661, but heat the sample to just above the melting point.

5.1.4.2 TEST PORTION

Weigh approximately 4 g of the test sample (see 8.5).

5.1.4.3 PREPARATION OF THE METHYL ESTERS (see 8.1)

Introduce the test portion into the flask (5.1.3.2). Add about 40 ml of the methanol (5.1.2.1), 0,5 ml of the methanolic potassium hydroxide solution (5.1.2.2) and a boiling aid (5.1.3.5). Fit the condenser (5.1.3.4) to the flask.

NOTE – In the presence of fatty acids containing more than two double bonds, it is recommended that the air in the methanolic solution and in the flask be removed by bubbling nitrogen (5.1.2.5) through the solution for a few minutes and maintaining a current of nitrogen into the upper part of the condenser during the following saponification.