SLOVENSKI STANDARD

SIST ISO 5508

prva izdaja
junij 1995

Rastlinske in živalske maščobe in olja - Določanje sestave maščobnih kislin z metodo plinske kromatografije (prevzet standard ISO 5508:1990 z metodo platnice)

Animal and vegetable fats and oils - Analysis by gas chromatography of methyl esters of fatty acids

Corps gras d'origines animale et végétale - Analyse par chromatographie en phase gazeuse des esters méthyliques d'acides gras

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Referenčna številka
SIST ISO 5508:1995 (en)

Nadaljevanje na straneh od II do III in 1 do 7

UVOD

Standard SIST ISO 5508, Rastlinske in živalske maščobe in olja - Določanje sestave maščobnih kislin z metodo plinske kromatografije, prva izdaja, 1995, ima status slovenskega standarda in je z metodo platnice prevzet mednarodni standard ISO 5508, Animal and vegetable fats and oils - Analysis by gas chromatography of methyl esters of fatty acids, second edition, 1990-09-15.

PREDGOVOR

Mednarodni standard ISO 5508:1990 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 5509 (en)	Rastlinske in živalske maščobe in olja - Priprava metil estrov maščobnih kislin
SIST ISO 5555 (en)	Rastlinske in živalske maščobe in olja - Vzorčenje

OSNOVA ZA IZDAJO STANDARDA

- Prevzem standarda ISO 5508:1990.
- Ta slovenski standard pokriva področje JUS E.K8.039:90.

OPOMBI

- Povsod, kjer se v besedilu standarda uporablja izraz "mednarodni standard", to pomeni v SIST ISO 5508:1995 "slovenski standard".

- Uvod in predgovor nista sestavni del standarda.

Po mnenju Ministrstva za informiranje Republike Slovenije z dne 18. februarja 1992, štev. 23/96-92, spada ta publikacija med proizvode informativne narave iz 13. točke tarifne številke 3, za katere se plačuje 5-odstotni prometni davek.

INTERNATIONAL STANDARD

ISO 5508

Second edition
1990-09-15

Animal and vegetable fats and oils — Analysis by gas chromatography of methyl esters of fatty acids

Corps gras d'origines animale et végétale — Analyse par chromatographie en phase gazeuse des esters méthyliques d'acides gras

Reference number
ISO 5508:1990(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 5508 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*.

This second edition cancels and replaces the first edition (ISO 5508:1978), of which it constitutes a technical revision.

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Animal and vegetable fats and oils — Analysis by gas chromatography of methyl esters of fatty acids

1 Scope

This International Standard gives general guidance for the application of gas chromatography, using packed or capillary columns, to determine the qualitative and quantitative composition of a mixture of fatty acid methyl esters obtained in accordance with the method specified in ISO 5509.

The method is not applicable to polymerized fatty acids.

2 Normative reference

The following standard contains provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the edition indicated was 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 edition of the standard indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 5509:1978, *Animal and vegetable fats and oils — Preparation of methyl esters of fatty acids.*

3 Reagents

3.1 Carrier gas

Inert gas (nitrogen, helium, argon, hydrogen, etc.), thoroughly dried and with an oxygen content of less than 10 mg/kg.

NOTE 1 Hydrogen, which is used as a carrier gas only with capillary columns, can double the speed of analysis but is hazardous. Safety devices are available.

3.2 Auxiliary gases

3.2.1 Hydrogen (purity ≥ 99,9 %), free from organic impurities.

3.2.2 Air or **oxygen**, free from organic impurities.

3.3 Reference standard

A mixture of methyl esters of pure fatty acids, or the methyl esters of a fat of known composition, preferably similar to that of the fatty matter to be analysed.

Care shall be taken to prevent the oxidation of polyunsaturated fatty acids.

4 Apparatus

The instructions given relate to the usual equipment used for gas chromatography, employing packed and/or capillary columns and a flame-ionization detector. Any apparatus giving the efficiency and resolution specified in 5.1.2 is suitable.

4.1 Gas chromatograph.

The gas chromatograph shall comprise the following elements.

4.1.1 Injection system.

Use an injection system either

a) with packed columns, having the least dead-space possible (in this case the injection system shall be capable of being heated to a temperature 20 °C to 50 °C higher than that of the column), or

b) with capillary columns, in which case the injection system shall be specially designed for use with such columns. It may be of the split type or it may be of the splitless on column injector type.

NOTE 2 In the absence of fatty acids with less than 16 carbon atoms, a moving needle injector may be used.

4.1.2 Oven.

The oven shall be capable of heating the column to a temperature of at least 260 °C and of maintaining the desired temperature to within 1 °C with a packed column and within 0,1 °C with a capillary column. The last requirement is particularly important when a fused silica tube is used.

The use of temperature-programmed heating is recommended in all cases, and in particular for fatty acids with less than 16 carbon atoms.

4.1.3 Packed column.

4.1.3.1 Column, constructed of a material inert to the substances to be analysed (i.e. glass or stainless steel) having the following dimensions.

a) Length: 1 m to 3 m. A relatively short column should be used when long-chain fatty acids (above C_{20}) are present. When analysing acids with 4 or 6 carbon atoms, it is recommended that a column 2 m in length is used.

b) Internal diameter: 2 mm to 4 mm.

NOTES

3 If polyunsaturated components with more than three double bonds are present, they may be decomposed in a stainless steel column.

4 A system with packed twin columns may be used.

4.1.3.2 Packing, comprising the following elements.

a) Support: Acid-washed and silanized diatomaceous earth, or other suitable inert support with a narrow range of grain size (25 µm range between the limits 125 µm to 200 µm), the average grain size being related to the internal diameter and length of the column.

b) Stationary phase: Polyester type of polar liquid (e.g. diethylene glycol polysuccinate, butanediol polysuccinate, ethyleneglycol polyadipate, etc.), cyanosilicones or any other liquid permitting the chromatographic separation required (see clause 5). The stationary phase should amount to 5 % (*m*/*m*) to 20 % (*m*/*m*) of the packing. A non-polar stationary phase can be used for certain separations.

4.1.3.3 Conditioning of the column.

With the column disconnected, if possible, from the detector, gradually heat the oven to 185 °C and pass a current of inert gas through the freshly prepared column at a rate of 20 ml/min to 60 ml/min for at least 16 h at this temperature, and for a further 2 h at 195 °C.

4.1.4 Capillary column.

4.1.4.1 Tube, made of a material inert to the substances to be analysed (usually glass or fused silica). The internal diameter shall be between 0,2 mm and 0,8 mm. The internal surface shall undergo an appropriate treatment (e.g. surface preparation, inactivation) before receiving the stationary phase coating. A length of 25 m is sufficient in most cases.

4.1.4.2 Stationary phase, usually of the type polyglycol [poly(ethylene glycol) 20 000], polyester (butanediol polysuccinate) or polar polysiloxane (cyanosilicones). Bonded (cross-linked) columns are suitable.

NOTE 5 There is a risk of polar polysiloxanes giving rise to difficulties in the identification and separation of linolenic acid and C_{20} acids.

The coatings shall be thin, i.e. 0,1 µm to 0,2 µm.

4.1.4.3 Assembly and conditioning of the column.

Observe the normal precautions for assembling capillary columns, i.e. arrangement of the column in the oven (support), choice and assembly of joints (leak tightness), positioning of the ends of the column in the injector and the detector (reduction of dead-spaces). Place the column under a flow of carrier gas [e.g. 0,3 bar (30 kPa) for a column of length 25 mm and internal diameter 0,3 mm].

Condition the column by temperature programming of the oven at 3 °C/min from ambient temperature to a temperature 10 °C below the decomposure limit of the stationary phase. Maintain the oven at this temperature for 1 h until stabilization of the baseline. Return it to 180 °C to work under isothermal conditions.

NOTE 6 Suitably pre-conditioned columns are available commercially.

4.1.5 Detector, preferably capable of being heated to a temperature above that of the column.

4.2 Syringe.

The syringe shall have a maximum capacity of 10 µl, and be graduated in 0,1 µl divisions.

4.3 Recorder.

If the recorder curve is to be used to calculate the composition of the mixture analysed, an electronic recorder of high precision, compatible with the apparatus used, is required. The recorder shall have the following characteristics:

a) rate of response, below 1,5 s, preferably 1 s (the rate of response is the time taken for the re-

cording pen to pass from 0 % to 90 % following the sudden introduction of a 100 % signal);

b) width of the paper, 20 cm minimum;

c) paper speed, adjustable to values between 0,4 cm/min and 2,5 cm/min.

4.4 Integrator or calculator (optional).

Rapid and accurate calculation can be performed with the help of an electronic integrator or calculator. This shall give a linear response with adequate sensitivity, and the correction for deviation of the base-line shall be satisfactory.

5 Procedure

The operations described in 5.1 to 5.3 relate to the use of a flame-ionization detector.

As an alternative a gas chromatograph employing a catharometer detector (working on the principle of thermal conductivity changes) may be used. The operating conditions are then modified as described in clause 7.

5.1 Test conditions

5.1.1 Selection of optimum operating conditions

5.1.1.1 Packed column

In the selection of the test conditions, the following variables should be taken into account:

a) the length and diameter of the column;

b) the nature and amount of the stationary phase;

c) the temperature of the column;

d) the carrier gas flow;

e) the resolution required;

f) the size of the test portion, selected in such a way that the assembly of the detector and electrometer gives a linear response;

g) the duration of analysis.

In general, the values given in table 1 and table 2 will lead to the desired results, i.e. at least 2 000 theoretical plates per metre of column length for methyl stearate and its elution within about 15 min.

Where the apparatus allows it, the injector should be at a temperature of about 200 °C and the detector at a temperature equal to or higher than that of the column.

As a rule, the ratio of the flow-rate of the hydrogen supplied to the flame-ionization detector to that of the carrier gas varies from 1:2 to 1:1 depending on the diameter of the column. The flow of oxygen is about 5 to 10 times that of the hydrogen.

Table 1

Internal diameter of column mm	Carrier gas flow ml/min
2	15 to 25
3	20 to 40
4	40 to 60

Table 2

Concentration of stationary phase % (*m*/*m*)	Column temperature °C
5	175
10	180
15	185
20	185

5.1.1.2 Capillary column

The properties of efficiency and permeability of capillary columns mean that the separation between constituents and the duration of the analysis are largely dependent on the flow-rate of the carrier gas in the column. It will therefore be necessary to optimize the operating conditions by acting on this parameter (or more simply on the headloss of the column), according to whether one wishes to improve the separations or to make a rapid analysis.

5.1.2 Determination of the number of theoretical plates (efficiency) and resolution

(See figure 1.)

Carry out the analysis of a mixture of methyl stearate and methyl oleate in about equivalent proportions (for example, methyl esters from cocoa butter).

Choose the temperature of the column and the carrier gas flow so that the maximum of the methyl stearate peak is recorded about 15 min after the solvent peak. Use a sufficient quantity of the mixture of methyl esters that the methyl stearate peak occupies about three-quarters of the full scale.