SLOVENSKI STANDARD
oSIST prEN ISO 12872:2022

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Oljčna olja in olja iz oljčnih tropin - Določevanje vsebnosti 2-gliceril monopalmitata (ISO/DIS 12872:2022)

Olive oils and olive-pomace oils - Determination of the 2-glyceryl monopalmitate content (ISO/DIS 12872:2022)

Olivenöle und Oliventresteröle – Bestimmung des Gehalts an 2-Glycerylmonopalmitat (ISO/DIS 12872:2022)

Huiles d'olive et huiles de grignons d'olive - Détermination de la teneur en 2-glycéryl monopalmitate (ISO/DIS 12872:2022)

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Olive oils and olive-pomace oils — Determination of the 2-glyceryl monopalmitate content

Huiles d'olive et huiles de grignons d'olive — Détermination de la teneur en 2-glycéryl monopalmitate

ICS: 67.200.10

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. 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.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 12872 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

Introduction

As part of the *Trade standard applying to olive oils and olive-pomace oils*, the International Olive Council (IOC) published COI/T.20/Doc. 23:2006[6]. COI/T.20/Doc. 23 was applicable to olive and olive-pomace oils and was used to distinguish between lampante virgin olive oils and crude olive-pomace oils. Olive pomace is the residual paste which still contains a variable amount of water and oil after pressing or centrifuging.

In 2008, the IOC submitted the document to ISO/TC 34/SC 11 for adoption as an International Standard.

In 2017, the IOC published a revision of the COI/T.20/Doc. n°23 (Rev.1), and this revised ISO document is an adoption of the IOC revised method.

Olive oils and olive-pomace oils — Determination of the 2-glyceryl monopalmitate content

1 Scope

This International Standard specifies a procedure for the determination of the content, as a percentage mass fraction, of 2-glyceryl monopalmitate in olive oils and olive-pomace oils that are liquid at ambient temperature (20 °C).

NOTE This International Standard is based on COI/T.20/Doc. n°23/Rev.1:2017[6].

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 661, *Animal and vegetable fats and oils — Preparation of test sample*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
2-glyceryl monopalmitate content
mass fraction of 2-glyceryl monopalmitate in the monoacylglycerol fraction, determined according to the method specified in this International Standard

Note 1 to entry: The 2-glyceryl monopalmitate content is expressed as a percentage.

4 Principle

The oil, after suitable preparation, is subjected to the action of pancreatic lipase. A partial hydrolysis takes place that is specific for positions 1 and 3 of the triacylglycerol molecule so that 2-monoacylglycerols are obtained as reaction products. The percentage of 2-glyceryl monopalmitate in the monoacylglycerol fraction is determined, after silylation, by capillary gas chromatography.

5 Reagents

WARNING — Comply with any local regulations which specify the handling of hazardous substances. Technical, organizational and personal safety measures shall be followed.

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade, and distilled or demineralized water or water of equivalent purity.

5.1 Silica gel, with a particle size of 0,063 mm to 0,200 mm (70/280 mesh), prepared as follows: put the silica gel into a porcelain cup, dry in an oven at 160 °C for 4 h, then cool at ambient temperature in a desiccator. Add a volume of water equivalent to 5 % of the mass of the silica gel as follows: weigh 152 g of silica gel into a 500 ml Erlenmeyer flask, add 8 g of water, stopper and homogenize carefully. Leave to settle for at least 12 h before using.

5.2 *n*-Hexane, chromatography grade.

NOTE Hexane may be replaced by iso-octane (2,2,4- trimethylpentane in chromatography grade), provided that comparable precision values are achieved (see Precision values of the method with the used of isooctane in Annex B).

5.3 Isopropanol.

5.4 Isopropanol-water mixture, volume fractions 50 ml/100 ml.

5.5 Pancreatic lipase, activity between 2,0 and 10 lipase units per milligram (see Annex C).

NOTE Pancreatic lipase with an activity between 2 and 10 units per mg of enzyme is available commercially.

5.6 Buffer solution of tris(hydroxymethyl)aminomethane: prepare an aqueous solution (1 mol/l) with pH 8 and mix with concentrated HCl, volume fractions 50 ml/100 ml.

5.7 Sodium cholate, special enzyme grade, aqueous solution, mass fraction 0,1 g/100 g.

Use this solution within 15 days of preparation.

5.8 Calcium chloride, aqueous solution, mass fraction 22 g/100 g.

5.9 Diethyl ether, chromatography grade.

5.10 Elution solvent 1: mixture of *n*-hexane-diethyl ether, volume fraction of *n*-hexane 87 ml/100 ml and of diethyl ether 13 ml/100 ml.

5.11 Sodium hydroxide, aqueous solution, mass fraction 12 g/100 g.

5.12 Phenolphthalein, ethanolic solution, mass concentration 1 g/100 ml.

5.13 Carrier gas: hydrogen or helium, gas chromatography grade.

5.14 Auxiliary gases: hydrogen, free from moisture and organic substances, and synthetic air, gas chromatography grade.

5.15 Silylation reagent: mixture of pyridine, hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS); volume fractions: 9 ml/13 ml, 3 ml/13 ml, and 1 ml/13 ml, respectively.

NOTE Ready-to-use solutions are available commercially. Other silylation reagents can be used, e.g. bis trimethylsilyl trifluoracetamide + 1 % trimethylchlorosilane, diluted with an identical volume of anhydrous pyridine.

5.16 Reference samples: pure monoacylglycerols and mixtures of monoacylglycerols with a known composition similar to that of the sample.

5.17 Elution solvent 2: mixture of *n*-hexane-diethyl ether, volume fraction of *n*-hexane 90 ml/100 ml and of diethyl ether 10 ml/100 ml.

6 Apparatus

Usual laboratory equipment and in particular the following.

6.1 Erlenmeyer flasks, of capacity 25 ml.

6.2 Beakers, of capacities 100 ml, 250 ml, and 300 ml.

6.3 Glass chromatography column, 21 mm to 23 mm internal diameter, 400 mm in length, with septum and stopcock.

6.4 Measuring cylinders, of capacities 10 ml, 50 ml, 100 ml, and 200 ml, ISO 4788[2] class A.

6.5 Round-bottomed flasks, of capacities 100 ml and 250 ml.

6.6 Rotary evaporator.

6.7 Centrifuge tubes, conical bottom, of capacity 10 ml, with ground-glass stopper.

6.8 Centrifuge, suitable for 10 ml and 100 ml tubes.

6.9 Water bath, capable of maintaining a temperature of (40 ± 0,5) °C.

6.10 Graduated pipettes, of capacities 1 ml and 2 ml, ISO 835[1] class A.

6.11 Hypodermic syringe, 1 ml.

6.12 Microsyringe, 100 µl.

6.13 Separating funnel, 1 000 ml.

6.14 Gas chromatograph, suitable for use with capillary columns, equipped with the components specified in 6.14.1 to 6.14.5.

6.14.1 Cold on-column injector.

6.14.2 Flame ionization detector.

6.14.3 Column oven, capable of maintaining the temperature to within ± 1 °C.

6.14.4 Computer-based integration system.

6.14.5 Fused silica capillary column, of length 8 m to 12 m and internal diameter 0,25 mm to 0,32 mm, coated with methylpolysiloxane or 5 % phenyl methylpolysiloxane, with a film thickness of 0,10 µm to 0,30 µm, suitable for use at 370 °C.

6.15 Microsyringe, 10 µl, with hardened needle, of minimum length 7,5 cm in length, suitable for on-column injection.

7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 5555[3].

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