INTERNATIONAL STANDARD

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Olive oils and olive-pomace oils — Determination of wax content by capillary gas chromatography

Huiles d'olive et huiles de grignons d'olive — Détermination de la teneur en cires par chromatographie en phase gazeuse sur colonne capillaire

Reference number
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Foreword

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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 12873 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 Oil Council (IOOC) — now known as International Olive Council (IOC) — published COI/T.20/Doc. 18:2007[4]. COI/T.20/Doc. 18 was applicable to olive and olive-pomace oils and was used to distinguish between oils obtained by either pressing or centrifuging and 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.

Olive oils and olive-pomace oils — Determination of wax content by capillary gas chromatography

1 Scope

This International Standard specifies the determination of the wax content, as a mass fraction expressed in milligrams per kilogram, of olive oils and olive-pomace oils. The individual waxes are separated according to the number of carbon atoms. The method is recommended for distinguishing between olive oil obtained by pressing or centrifuging and that obtained from olive pomace (olive-pomace oil).

NOTE This International Standard is based on COI/T.20/Doc. 18/Rev. 2:2007[4].

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
wax content
mass fraction of those substances in a sample determined in accordance with the method specified in this International Standard

NOTE The wax content is expressed in milligrams per kilogram.

4 Principle

After a suitable internal standard has been added, the oil is fractionated by chromatography on a hydrated silica gel column. The fraction eluted under test conditions (which has a lower polarity than the triglycerides) is recovered and analysed 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 60 µm to 200 µm, prepared as follows: place the gel in the muffle oven at 500 °C for at least 4 h; allow to cool, then add 2 % of water in relation to the mass of silica gel used; shake well to homogenize the slurry. Store in darkness for at least 12 h prior to use.

5.2 *n*-Hexane, chromatography grade.

5.3 Diethyl ether, chromatography grade.

5.4 *n*-Heptane, chromatography grade.

5.5 Internal standard, solution of lauryl arachidate in hexane, mass concentration 0,1 g/100 ml.

NOTE Palmityl palmitate or myristyl stearate may also be used.

5.6 Sudan I (1-phenylazo-2-naphthol).

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

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

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Erlenmeyer flask, 25 ml.

6.2 Glass column for liquid chromatography, of diameter 15,0 mm and length 30 cm to 40 cm, with stopcock.

6.3 Gas chromatograph, suitable for use with a capillary column, equipped with the components specified in 6.3.1 to 6.3.5.

6.3.1 Cold on-column injector.

6.3.2 Thermostat-controlled oven with temperature programming.

6.3.3 Flame-ionization detector.

6.3.4 Computer-based integration system.

6.3.5 Capillary column, fused silica, of length 8 m to 12 m and internal diameter 0,25 mm to 0,32 mm, with SE-52 or SE-54[1)] liquid phase or equivalent, with a film thickness of 0,10 µm to 0,30 µm.

6.4 Microsyringe for on-column injection, of capacity 10 µl, with a hardened needle.

6.5 Electric shaker.

6.6 Rotary evaporator.

6.7 Muffle oven.

6.8 Analytical balance, for weighing to an accuracy of 0,1 mg.

1) SE-52 and SE-54 are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 5555[1].

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

8 Preparation of the test sample

Prepare the test sample in accordance with ISO 661.

9 Procedure

9.1 Preparation of the chromatography column

Suspend 15 g of silica gel (5.1) in *n*-hexane (5.2) and introduce into the chromatography column (6.2). Allow to settle spontaneously. Use an electric shaker (6.5) to assist in complete settling to make the chromatographic band more homogeneous. Percolate 30 ml *n*-hexane to remove any impurities. Weigh, to the nearest 0,1 mg, about 500 mg of the sample into a 25 ml Erlenmeyer flask (6.1), add a suitable amount of internal standard (5.5) depending on the assumed wax content. Add 0,1 mg of lauryl arachidate in the internal standard solution (5.5) in the case of olive oil, and 0,25 mg to 0,50 mg in the case of olive-pomace oil.

Transfer the test portion to the chromatographic column with the aid of two 2 ml portions of *n*-hexane.

Allow the solvent to drain off to 1 mm above the upper level of the absorbent. Percolate a further 70 ml of *n*-hexane to remove any *n*-alkanes naturally present. Then start chromatographic elution by collecting 180 ml of a mixture of 99 ml/100 ml *n*-hexane (5.2) and 1 ml/100 ml diethyl ether (5.3) at a rate of about 15 drops every 10 s. Perform the column chromatography at room temperature.

Prepare the *n*-hexane-diethyl ether mixture freshly every day.

To check visually that the waxes have been completely eluted, add 100 µl of Sudan I dye (5.6) solution at a concentration of 1 g/100 ml to the test portion solution. The dye is retained on the chromatographic column between the waxes and triglycerides. Hence, when the dye reaches the bottom of the column, suspend elution because all the waxes have been eluted.

Evaporate the resultant fraction in a rotary evaporator (6.6) until the solvent is almost removed. Remove the last 2 ml of solvent under a weak stream of nitrogen, then add 2 ml to 4 ml of *n*-heptane.

9.2 Gas chromatographic analysis

9.2.1 Preliminary procedure

If the column is being used for the first time, it is advisable to condition it. Run a light flow of gas through the column, then switch on the gas chromatograph. Heat gradually for approximately 4 h until a temperature of 350 °C is reached.

Maintain this temperature for at least 2 h, then regulate the apparatus to the operating conditions (gas flow, light flame, oven temperature for column, detector, etc.). Record the signal at a sensitivity that is at least twice as high as that required for the analysis. The baseline should be linear, with no peaks of any kind, and shall not drift. Negative straight-line drift indicates incorrect column connections, while positive drift is symptomatic of improper column conditioning.

9.2.2 Operating conditions

The following operating conditions are recommended.

— Column temperature:

  — isothermal at 80 °C for 1 min,

  — increase at a rate of 20 °C/min to 240 °C,

  — increase at a rate of 5 °C/min to 325 °C; 5 min isothermal at 325 °C,

  — increase at a rate of 20 °C/min to 340 °C; 10 min isothermal at 340 °C;

— detector temperature: 350 °C;

— injection volume: 1 µl of *n*-heptane solution;

— carrier gas: helium or hydrogen at the optimal linear speed for the gas chosen (see Annex C).

Due to the high final column temperature of 340 °C, a positive drift is permissible, but may not exceed more than 10 % of the full-scale value.

These conditions shall be modified to suit the characteristics of the column and the gas chromatograph in order to separate all the waxes and to obtain satisfactory peak separation (see Figure A.1). The retention time of the internal standard shall be (18 ± 3) min and the most representative peak of the waxes shall be over 60 % of the full-scale value.

9.3 Performance of the analysis

Take up 1 µl of the solution using the 10 µl microsyringe (6.4); draw back the plunger until the needle is empty. Introduce the needle into the injection system and inject quickly after 1 s to 2 s. After about 5 s, gently remove the needle. Record the chromatogram until the waxes are completely eluted. The baseline shall always satisfy the required conditions.

9.4 Peak identification

Identify the peaks from the retention times by comparing them with mixtures of waxes with known retention times, analysed under the same conditions.

Figure A.1 shows a chromatogram of the wax fraction of a virgin olive oil.

10 Quantitative analysis and expression of results

Determine the areas of the peaks corresponding to the internal standard and the aliphatic esters from C_{40} to C_{46} and calculate the wax content, w_W, in milligrams per kilogram of oil, according to the equation:

$$w_W = \left(\sum_i \frac{A_i \; m_{IS}}{A_{IS} \; m} \right) \times 1\,000$$

where

A_i is the peak area of each ester peak from C_{40} to C_{46} inclusive;

A_{IS} is the peak area of the internal standard peak;

m_{IS} is the mass, in milligrams, of the internal standard added;

m is the mass, in grams, of the test portion.

Report the sum of the contents of the different waxes from C_{40} to C_{46}, in milligrams per kilogram, to one decimal place.

The components for quantification refer to the peaks with even carbon numbers amongst the esters C_{40} to C_{46} (see chromatogram of the wax fraction of olive oil in Figure A.1). If the C_{46} ester peak is split, it is recommended that the wax fraction of an olive-pomace oil be analysed, where the C_{46} peak is the predominant peak.

11 Precision

11.1 Interlaboratory test

Details of an interlaboratory test on the precision of the method are summarized in Annex B. The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given.

11.2 Repeatability

The absolute difference between two independent single test results, obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, should, in not more than 5 % of cases, exceed the values of the repeatability limit, r, given in Table B.1.

11.3 Reproducibility

The absolute difference between two single test results, obtained with the same method on identical test material in different laboratories by different operators using different equipment, should, in not more than 5 % of cases, exceed the values of the reproducibility limit, R, given in Table B.1.

12 Test report

The test report shall include at least the following information:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

c) the test method used, with reference to this International Standard (ISO 12873:2010);

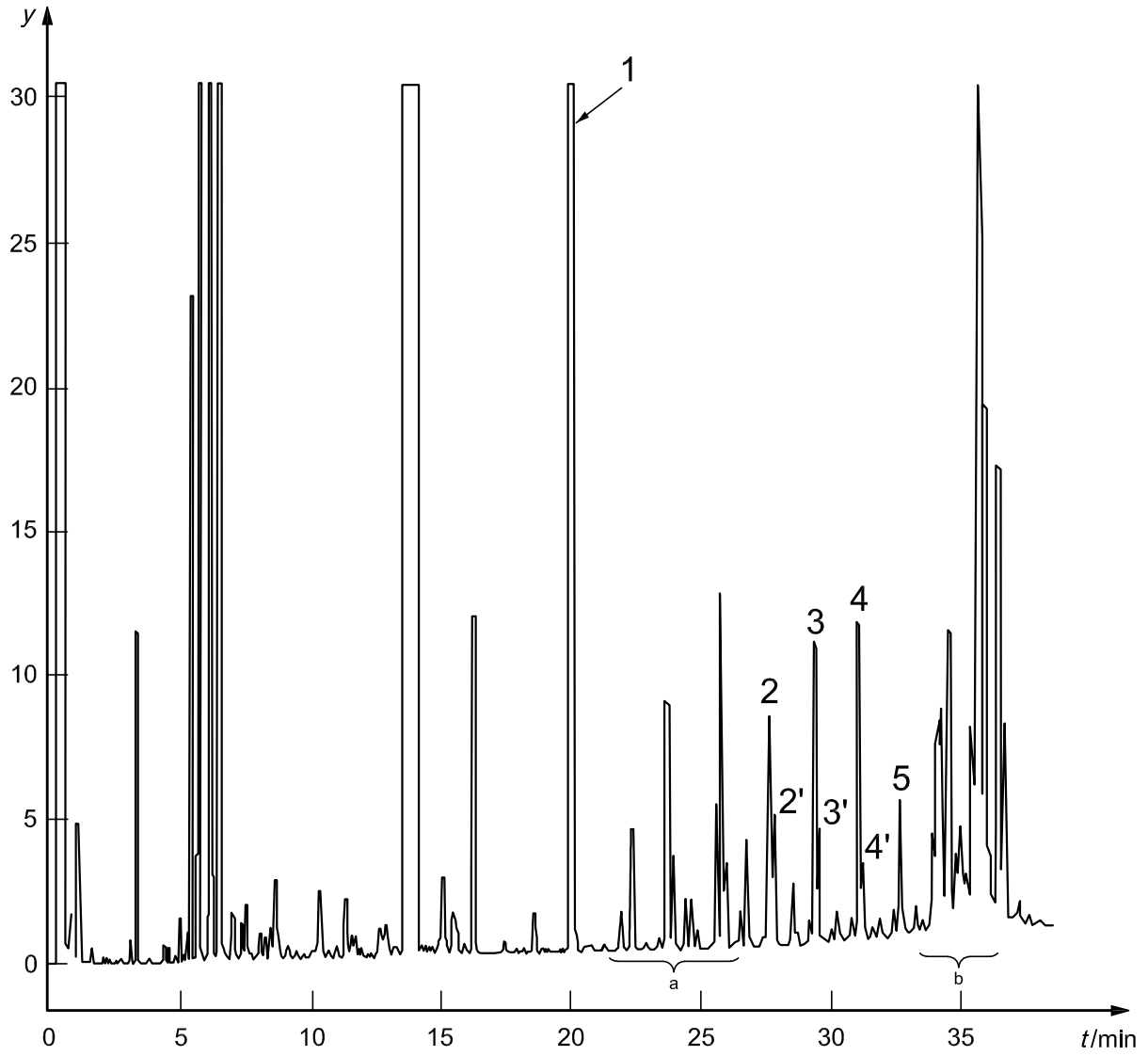
d) the result(s) obtained;

e) if the repeatability has been checked, the final quoted result obtained;

f) any operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s).

Annex A
(informative)

Chromatogram



Key

1	lauryl arachidate	5	C_{46} esters
2, 2′	C_{40} esters	t	time
3, 3′	C_{42} esters	y	measure of relative peak size, arbitrary units
4, 4′	C_{44} esters		

NOTE After elution of the sterol esters[b], the gas chromatogram shall not show any significant peaks (triglycerides).

[a] Diterpenic esters.

[b] Sterol esters and triterpenic alcohols.

Figure A.1 — Chromatogram of the wax fraction of olive oil

Annex B
(informative)

Results of an interlaboratory test

The precision data in Table B.1 are derived from the results of an international collaborative trial.

The interlaboratory test was organized by the Executive Secretariat of the International Olive Council in 1999. Participants included 19 laboratories holding IOC recognition from eight countries. The test was performed on five samples.

The test results were subjected to statistical analysis in accordance with ISO 5725-1[2] and ISO 5725-2[3] to give the precision data shown in Table B.1. Outliers were examined by applying the tests of Cochran and Grubbs.

Table B.1 — Statistical results

Parameter	Sample				
	A Extra virgin olive oil	**B** Virgin olive oil/refined sunflower oil	**C** Virgin olive oil/refined olive-pomace oil	**D** Virgin olive oil/refined soybean oil/refined sunflower oil	**E** Refined olive oil/refined olive-pomace oil/refined soybean oil/lampante virgin olive oil
No. participating laboratories, n_P	19	19	19	19	19
No. laboratories retained after eliminating outliers, n_p	14	14	15	16	14
No. test results in all laboratories, n_t	38	38	38	38	38
Mean wax content, $\bar{w}_w$, mg/kg	120,32	123,14	222,41	174,10	345,93
Repeatability standard deviation, s_r, mg/kg	3,39	4,48	3,75	4,72	5,32
Coefficient of variation of repeatability, $C_{V,r}$, %	2,8	3,6	1,7	2,7	1,5
Repeatability limit, r, mg/kg	9,51	12,56	10,51	12,22	14,91
Reproducibility standard deviation, s_R, mg/kg	13,86	17,46	21,04	9,16	15,85
Coefficient of variation of reproducibility, $C_{V,R}$, %	11,5	14,2	9,5	5,3	4,6
Reproducibility limit, R, mg/kg	38,83	48,89	58,93	25,65	44,39

Annex C
(informative)

Determination of the linear velocity of the gas

Inject 1 µl to 3 µl of methane or propane into the gas chromatograph at normal operating conditions and register the elution time, t, for methane or propane.

The linear velocity, in centimetres per second, is given by

$$v = \frac{L}{t}$$

where

L is the length, in centimetres, of the column;

t is the measured time, in seconds.

Bibliography

[1] ISO 5555, *Animal and vegetable fats and oils — Sampling*

[2] ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

[3] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

[4] COI/T.20/Doc. 18:2007, *Method of analysis: Determination of wax content by capillary column gas chromatography*

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