DRAFT INTERNATIONAL STANDARD

ISO/DIS 23942

ISO/TC **34**/SC **11**	Secretariat: **BSI**
Voting begins on: **2019-12-17**	Voting terminates on: **2020-03-10**

Determination of hydroxytyrosol and tyrosol content in extra virgin olive oils — HPLC method

ICS: 67.200.10

This document is circulated as received from the committee secretariat.

Reference number
ISO/DIS 23942:2019(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee /TC *34 Food Products* Subcommittee SC 11, *Animal and vegetable fats and oils.*

Introduction

The biophenolic compounds of secoiridoid nature and peculiar of extra virgin olive oil (Olea europaea L.) are derived from oleuropein and ligstroside and are correlated to different beneficial health effects on human being other than particular sensorial characteristics. The biophenolic compounds contain as esterified form two aromatic alcohols, namely hydroxytyrosol and tyrosol. The method is based on extraction of the biophenolic fraction with a methanol/water solution and a subsequent hydrolysis reaction to produce free tyrosol and hydroxytyrosol[1][2].

Determination of hydroxytyrosol and tyrosol content in extra virgin olive oils — HPLC method

1 Scope

This International Standard specifies a method for the quantitative determination of hydroxytyrosol and tyrosol content in extra virgin olive oils using reverse phase (RP) HPLC with spectrophotometric detectotion. The method is also applicable to all other olive oils of different commercial category.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org/obp

3.1
Hydroxytyrosol and tyrosol
total content expressed as sum of mg/kg of hydroxytyrosol and tyrosol in extra virgin olive oils after extraction from the oil and hydrolysis reaction to the free form.

4 Principle

Hydroxytyrosol and tyrosol, present in free and esterified forms, are extracted from the oil with a methanol/water solution and then submitted to hydrolysis reaction with a 10% of sulphuric acid ethanolic solution. The components are identified by means of HPLC and spectrophotometric detector at 280 nm and the amount of free aromatic alcohols is calculated with the use of an external standard.

5 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade.

5.1 Ortophosphoric acid 85 % v/v

5.2 Methanol chromatographic grade

5.3 Acetonitrile chromatographic grade

5.4 Water chromatographic grade

5.5 Ethanol 96 % v/v

5.6 Sulphuric acid 96 % v/v

5.7 Methanol/Water solution 80/20 v/v

5.8 Reference sample: hydroxytyrosol or 2-(3,4-Dihydroxyphenyl)ethanol e.g. Extrasynthese Cedex France and Tyrosol e.g. Sigma Aldrich Germany[1)]

5.9 Reference sample Tyrosol e.g. Sigma Aldrich Germany

5.9.1 External standard calibration solution of hydroxytyrosol and tyrosol preparation

Weigh exactly 25 mg of hydroxytyrosol (5.7) and tyrosol (5.8) in a graduated 50 ml flask (6.2) and take to volume with a solution of methanol/water 80/20 v/v (5.7). Transfer 1 ml (5.3) in another 10 ml flask and fill to volume with the same solution (5.7). The final concentration will be 50 mg/l of each external standard. Inject 20 µl of this solution in the HPLC system. The solution is stable for 6 months at least at – 20°C.

5.10 Hydrolysis solution constituted by ethanol/water/sulphuric acid 50/40/10 v/v/v.

6 Apparatus

Usual laboratory glassware

6.1 Analytical balance suitable to perform weighing to an accuracy of within +/- 0,1 mg

6.2 10 and 50 ml calibrated flasks class A

6.3 1000 µl and 5000 µl electronic pipette or manual pipette

6.4 10 ml test tube with screw cap

6.5 Mixer type Vortex

6.6 Ultrasonic extraction bath

6.7 PVDF (Polyvinyl difluoride) Syringe filters 0,45 µm, 13 mm

6.8 Centrifuge able to operate at 5000 rpm

6.9 5 ml plastic syringe

6.10 Thermostatic bath

6.11 Analytical system comprising of a HPLC ternary pump equipped with HPLC column, RP 18 reverse phase. The following column has proven to be adapted for the determination (internal diameter 4,6 mm, length 25 cm, size 5 µm, 100 A°, type Spherisorb ODS2[2)]**) with a UV spectrophotometric detector at 280 nm and integration system. The possibility to use PDA for spectra recording may facilitate the peak identification**

1) Extrasynthese Cedex France and Sigma Aldrich is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

2) The Spherisorb ODS2 column is an example of chromatographic column suitable and commercially available. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

7 Sampling

It is important an intact oil sample is delivered to the laboratory, no damaged or modified during transport or storage. A representative sample is considered for the purpose of the analysis. A recommended sampling method is reported in EN ISO 5555[8].

8 Procedure

8.1 Sample preparation

Weigh with an analytical balance (6.1) 2 g of oil well homogenized in a 10 ml conical test tube (6.4). Add (6.3) 5 ml of a methanol/water solution 80/20 v/v (5.7). Mix the solution with the help of a mixer for test tube type Vortex (6.5) for 1 minute and continue the extraction for 15 minutes in an ultrasonic bath (6.6) at room temperature. Centrifuge (6.8) at 5000 rpm for 25 minutes. An aliquot is filtered through a PVDF membrane syringe filter (6.7), transfer 1 ml (6.3) of the filtered solution in another 10 ml test tube (6.4) to be completely dried on a thermostatic bath (6.10) at a maximum temperature of 40°C under nitrogen stream. 1 ml of hydrolysis solution (5.10) is added and mixed followed by reaction at 40° C for 1 hour. The solution is left at room temperature for a whole night. The solution is filtered using a PVDF membrane syringe filter (6.7).

8.2 HPLC Analysis

20 µl of sample is injected onto the HPLC system (6.11). The first sample injected in a series of analysis shall be a blank of a methanol/water solution 80/20 v/v (5.7). There must be no interfering signals present during the chromatographic run at the same retention time of hydroxytyrosol and tyrosol.

8.2.1 HPLC conditions

The following operating conditions have proven to be adapted for the determination:

Time min	Flow ml/min	A %	B %	C %
0	1.00	96	2	2
40	1.00	50	25	25
45	1.00	40	30	30
60	1.00	0	50	50
70	1.00	0	50	50
72	1.00	96	2	2
82	1.00	96	2	2
A= water 0.2 % H_3PO_4; B=methanol; C=acetonitrile				

8.2.2 The ternary gradient is programmed to enable observation in the full chromatogram that hydrolysis is complete with no bound forms remaining in the extract. Once the operators are experienced with the analysis, the elution time could be reduced by stopping the gradient after elution of tyrosol, followed by washing the column for ten minutes with B/C solvents in ratio of 50/50 v/v and then reconditioning for 10 minutes with A/B/C solvents 96/2/2 v/v/v.

The solvents for the elution must first be degassed.

The spectrophotometric detector (280 nm) must be turned on an hour before the first analysis. The HPLC column must be conditioned for at least 15 min before the gradient development with the initial