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Determination of hydroxytyrosol and tyrosol content in extra virgin olive oils — Reverse phase high performance liquid chromatography (RP-HPLC)

Détermination de la teneur en hydroxytyrosol et tyrosol dans les huiles d'olive vierges extra — Chromatographie liquide à haute performance en phase inverse (CLHP-RP)

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Foreword

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

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Biophenolic compounds of a secoiridoid nature and typical of extra virgin olive oil (*Olea europaea* L.), are derived from oleuropein and ligstroside and are correlated to different beneficial health effects for human beings other than particular sensorial characteristics. The biophenolic compounds contain, in an esterified form, two aromatic alcohols, namely hydroxytyrosol and tyrosol. The method given in this document is based on an 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 — Reverse phase high performance liquid chromatography (RP-HPLC)

1 Scope

This document specifies a method for the quantitative determination of hydroxytyrosol and tyrosol content in extra virgin olive oils using reverse phase high performance liquid chromatography (RP-HPLC) with spectrophotometric detection. The method is also applicable to all other olive oils of a 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:

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

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

3.1
hydroxytyrosol and tyrosol
aromatic alcohols present in extra virgin olive oil typical of *Olea europaea* L. species as free or bound 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 a spectrophotometric detector at 280 nm. 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 Ortho-phosphoric acid, a volume fraction of 85 %.

5.2 Methanol chromatographic grade.

5.3 Acetonitrile chromatographic grade.

5.4 Water chromatographic grade.

5.5 Ethanol, a volume fraction of 96 %.

5.6 Sulphuric acid, a volume fraction of 96 %.

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)[1)].

5.9 Reference sample: tyrosol, e.g. Sigma Aldrich (Germany)[1)].

5.10 Standard calibration solution of hydroxytyrosol and tyrosol.

Prepare the external standard calibration solution of hydroxytyrosol and tyrosol as follows.

Weigh accurately, to the nearest 0,1 mg, about 25 mg of hydroxytyrosol (5.8) and tyrosol (5.9) in a graduated 50 ml flask (6.2) and make to volume with a solution of methanol/water 80/20 v/v (5.7). Transfer 1 ml of this solution in another 10 ml flask and fill to volume with the same solution of methanol/water 80/20 v/v (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 at least six months at –20 °C.

5.11 Hydrolysis solution, consisting of ethanol/water/sulphuric acid 50/40/10 v/v/v.

6 Apparatus

Usual laboratory glassware and the following.

6.1 Analytical balance suitable for weighing to an accuracy of within ±0,1 mg.

6.2 10 ml and 50 ml calibrated flasks, class A.

6.3 1 000 µl and 5 000 µl electronic pipette or manual pipette.

6.4 10 ml test tube, with a 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 5 000 r/min.

6.9 5 ml plastic syringe.

6.10 Thermostatic bath.

6.11 Analytical system, comprising a HPLC ternary pump with a degassing system equipped with an HPLC column, RP 18 reverse phase. The following column has proven to be adapted for the

1) Extrasynthese (Cedex, France) and Sigma Aldrich (Germany) are examples of a companies that make 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.

determination (internal diameter 4,6 mm, length 25 cm, size 5 µm, 100 Å, type Spherisorb ODS2[2)]) with a UV spectrophotometric detector at 280 nm and integration system. A photodiode array detector (PDA) for spectra recording could be used to facilitate peak identification, by matching the hydroxytyrosol and tyrosol spectra in the sample extract with the spectra of the external standard.

A system for analysis data and integration is needed.

7 Sampling

It is important that an intact oil sample is delivered to the laboratory, which has not been damaged or modified during transport or storage. A representative sample is considered for the purpose of the analysis. A recommended sampling method is given in ISO 5555.

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, using a pipette (6.3), 5 ml of the 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 min and continue the extraction for 15 min in an ultrasonic bath (6.6) at room temperature. Centrifuge (6.8) at 5 000 r/min for 25 min. Filter an aliquot through a PVDF membrane syringe filter (6.7). Transfer 1 ml, using a pipette (6.3), of the filtered solution into another 10 ml test tube (6.4) and completely dry on a thermostatic bath (6.10) at a maximum temperature of 40° C under nitrogen stream. Add 1 ml of hydrolysis solution (5.11) and mix, followed by reaction at 40° C for 1 h. Leave the solution at room temperature overnight. Then filter the solution using a PVDF membrane syringe filter (6.7).

8.2 HPLC analysis

8.2.1 General

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

8.2.2 HPLC conditions

The operating conditions given in Table 1 have proven to be adapted for the determination.

2) The Spherisorb ODS2 column is an example of suitable chromatographic column that is 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.