Αποστολή με ηλεκτρονικό μήνυμα

ΕΛΛΗΝΙΚΗ ΔΗΜΟΚΡΑΤΙΑ
ΥΠΟΥΡΓΕΙΟ ΑΓΡΟΤΙΚΗΣ ΑΝΑΠΤΥΞΗΣ & ΤΡΟΦΙΜΩΝ

ΕΦΕΤ

ΕΘΝΙΚΟΣ ΦΟΡΕΑΣ ΕΛΕΓΧΟΥ ΤΡΟΦΙΜΩΝ

Αθήνα, 06.02.2018

Αρ. Πρωτ.: 1654

Δ/ΝΣΗ ΑΞΙΟΛΟΓΗΣΗΣ - ΕΓΚΡΙΣΕΩΝ

Δ/ΝΣΗ ΕΡΓΑΣΤΗΡΙΑΚΩΝ ΕΛΕΓΧΩΝ

Ταχ. Δ/νση: Λεωφ. Κηφισίας 124 & Ιατρίδου 2
Ταχ. Κώδικας 115 26, Αμπελόκηποι
Πληροφορίες Ασπασία Σαμωνά, Σταυρούλα Σκούλικα
Τηλέφωνο: 213-2145826, 210-2725314
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ΠΡΟΣ: Λίστα Αποδεκτών

ΘΕΜΑ: **«Ενημέρωση σχετικά με τη χρήση του ισχυρισμού υγείας του Καν.(ΕΕ) αριθ. 432/2012 για τις πολυφαινόλες ελαιολάδου»**

ΣΧΕΤ.: **Απόσπασμα πρακτικού 16/4-12-2017 του Δ.Σ του Ε.Φ.Ε.Τ. (ΑΔΑ:ΩΡΟ0ΟΡ9Τ-Ι8Ω)**

Το Διοικητικό Συμβούλιο του ΕΦΕΤ με την υπ΄αριθ. 414/4-12-2017 Απόφασή του υιοθέτησε την απόφαση-εισήγηση του Επιστημονικού Συμβουλίου Ελέγχου Τροφίμων (ΕΣΕΤ) του Ε.Φ.Ε.Τ. μετά από σχετική πρόταση της αντίστοιχης ομάδας εργασίας σχετικά με τον ισχυρισμό υγείας του Παραρτήματος του Κανονισμού (ΕΕ) αριθ. 432/2012 για τις πολυφαινόλες ελαιολάδου, ήτοι: «*Οι πολυφαινόλες ελαιολάδου συμβάλλουν στην προστασία των λιπιδίων του αίματος από το οξειδωτικό στρες*» συμπεριλαμβανόμενης της λίστας των ουσιών και των μεθοδολογιών στις οποίες κατέληξε η Ομάδα Εργασίας του ΕΣΕΤ.

Συγκεκριμένα, σύμφωνα με την ανωτέρω απόφαση για την τεκμηρίωση του ισχυρισμού υγείας για τις πολυφαινόλες ελαιολάδου μπορούν να προσμετρώνται η υδροξυτυροσόλη, η τυροσόλη και τα παράγωγά τους, όπως αυτά απαριθμούνται στο επισυναπτόμενο Παράρτημα Ι.

Στο Παράρτημα ΙΙ αναφέρονται οι μέθοδοι που μπορούν να χρησιμοποιηθούν για τον προσδιορισμό των ανωτέρω ενώσεων, ενώ για τον επίσημο έλεγχο του εν λόγω ισχυρισμού υγείας από τις αρμόδιες Αρχές Ελέγχου Τροφίμων, και στο παρόν πλαίσιο, αποφασίστηκε η χρήση της μεθόδου του Διεθνούς Συμβουλίου Ελαιοκομίας - COI/T.20/Doc No 29, November 2009, σύμφωνα και με την εισήγηση της Ομάδας Εργασίας.

Σημειώνεται ότι τα Παραρτήματα Ι και ΙΙ επικαιροποιούνται κάθε φορά που προκύπτουν νέα στοιχεία.

Τέλος, επισημαίνεται ότι την ευθύνη της χρήσης του ανωτέρω ισχυρισμού φέρει ο εκάστοτε υπεύθυνος επιχείρησης τροφίμων.

Στη διάθεσή σας για οποιαδήποτε πληροφορία,

Ο Πρόεδρος του ΔΣ του ΕΦΕΤ

ΕΛΛΗΝΙΚΗ ΔΗΜΟΚΡΑΤΙΑ ΥΠΟΥΡΓΕΙΟ ΑΓΡΟΤΙΚΗΣ ΑΝΑΠΤΥΞΗΣ & ΤΡΟΦΙΜΩΝ ΕΦΕΤ

ΑΚΡΙΒΕΣ ΑΝΤΙΓΡΑΦΟ

06-02-2018

Κων/νος Τατάκης

Ιωάννης Τσιάλτας

ΠΙΝΑΚΑΣ ΑΠΟΔΕΚΤΩΝ

Αποδέκτες προς ενέργεια

1. Περιφερειακές Δ/σεις Ε.Φ.Ε.Τ.
2. Περιφέρειες της Χώρας για ενημέρωση όλων των αρμοδίων υπηρεσιών των Περιφερειών και των Περιφερειακών Ενοτήτων
3. Εργαστήρια Δοκιμών & Ερευνών Τροφίμων Ε.Φ.Ε.Τ.
4. Ανεξάρτητη Αρχή Δημοσίων Εσόδων

   Γενική Δ/νση Γενικού Χημείου του Κράτους

   Δ/νση Αλκοόλης & Τροφίμων
5. Υπουργείο Οικονομίας & Ανάπτυξης

   Γενική Γραμματεία Εμπορίου & Προστασίας Καταναλωτή

   Γενική Δ/νση Αγοράς

   Δ/νση Ελέγχων & Παρατηρητηρίων

   Τμήμα Χημικών Αναλύσεων

Αποδέκτες προς κοινοποίηση

1. Υπουργείο Αγροτικής Ανάπτυξης & Τροφίμων:

   Α) Γραφείο Υπουργού κου Αποστόλου

   Β) Γραφείο Αναπληρωτή Υπουργού κου Τσιρώνη

   Γ) Γραφείο Υφυπουργού κου Κόκκαλη

   Δ) Γραφείο Γενικού Γραμματέα κου Αντώνογλου

   Ε) Γραφείο Γενικού Γραμματέα Αγροτικής Πολιτικής και Διαχείρισης Κοινοτικών Πόρων κου Κασίμη

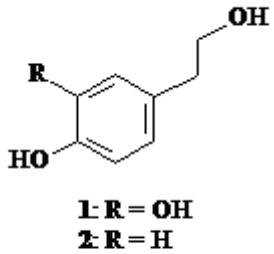
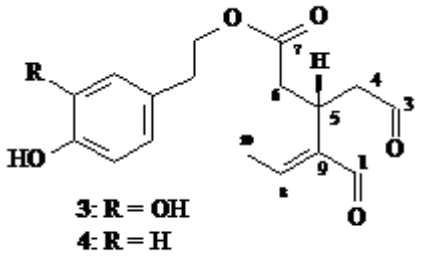
   Στ)Γενική Δ/νση Τροφίμων

   Ζ)Γενική Δ/νση Γεωργίας
2. ΕΛΓΟ-ΔΗΜΗΤΡΑ
3. Εθνική Διεπαγγελματική Οργάνωση Ελαιολάδου & Ελιάς (ΕΔΟΕΕ)
4. Σύνδεσμος Ελληνικών Βιομηχανιών Τυποποιήσεως Ελαιολάδου (ΣΕΒΙΤΕΛ)
5. Πανελλήνιος Σύνδεσμος Ελαιουργείων (ΠΑ.Σ.ΕΛ)
6. Ελληνικό Κέντρο Εξαγωγών και Προώθησης Ελαιολάδου (ΕΚΕΠΕ)
7. Σύνδεσμος Ελληνικών Βιομηχανιών τροφίμων (ΣΕΒΤ)
8. Γραμματεία ΕΣΕΤ για ενημέρωση των μελών του ΕΣΕΤ και των μελών της σχετικής Ομάδας Εργασίας του ΕΣΕΤ

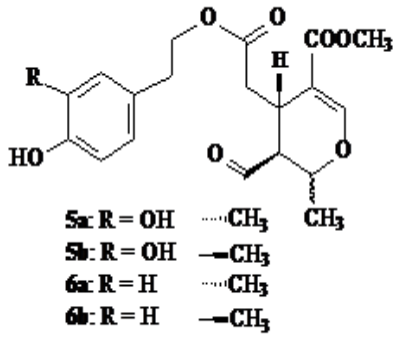
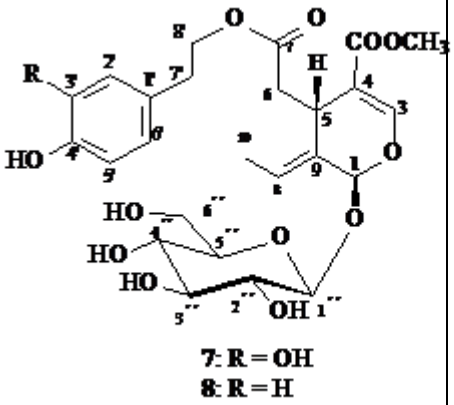
Εσωτερική Διανομή:

1. Γραφείο Προέδρου Δ.Σ Ε.Φ.Ε.Τ.
2. Γραφείο Αντιπροέδρου Δ.Σ Ε.Φ.Ε.Τ.
3. Δ/σεις ΚΥ Ε.Φ.Ε.Τ.

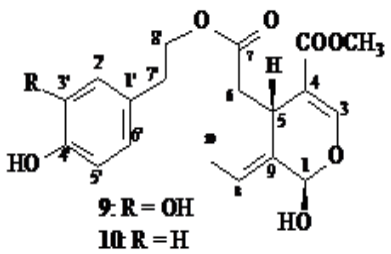
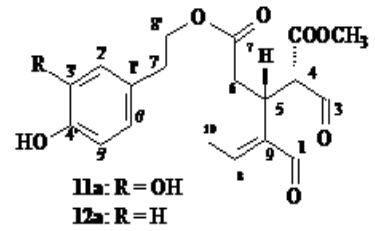
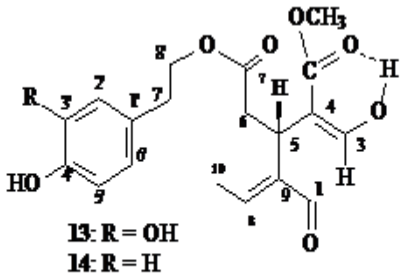
ΠΑΡΑΡΤΗΜΑ Ι

Δομές υδροξυτυροσόλης, τυροσόλης και συγγενών δομικά ενώσεων που έχουν αναφερθεί στη βιβλιογραφία με διάφορες μεθόδους και που εν δυνάμει συνεισφέρουν στον ισχυρισμό υγείας για τις φαινόλες του ελαιολάδου (Καν 432/12)				
Συντακτικός τύπος	**Ονομασία**	**Εμπειρική ονομασία**	**Ονομασία/ες στην Αγγλική**	**Συντομογραφία**
 **1**: R = OH **2**: R = H	**1:** 2-(3,4-διυδροξυφαινυλο)-αιθανόλη **2**: 2-(4-υδροξυφαινυλο)-αιθανόλη	**1:** υδροξυτυροσόλη **2**: τυροσόλη	**1: (**3,4-dihydroxyphenyl) ethanol/ hydroxytyrosol **2**: (p -hydroxyphenyl) ethanol/ tyrosol	**1:** 3,4-DHPEA **2**: p-HPEA
 **3**: R = OH **4**: R = H	**3**: Διαλδεϋδική, αποκαρβοξυμεθυλιωμένη μορφή του άγλυκου της ελαιοευρωπαΐνης **4:** Διαλδεϋδική, αποκαρβοξυμεθυλιωμένη μορφή του άγλυκου του λιγκστροζίτη	**3**: ελαιασίνη **4:** ελαιοκανθάλη	**3**: Dialdehydic form of decarboxymethyl elenolic acid linked to 3,4-DHPEA/oleacein **4:** dialdehydic form of decarboxymethyl elenolic acid linked to p –HPEA/oleocanthal	3,4-DHPEA-EDA p-HPEA-EDA

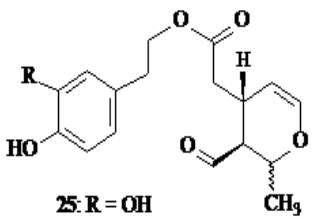
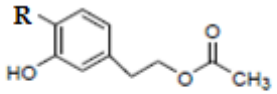
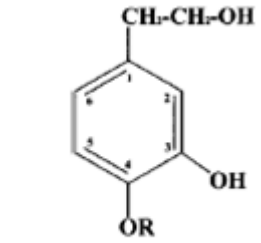
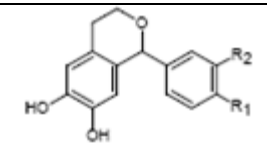
ΠΑΡΑΡΤΗΜΑ Ι

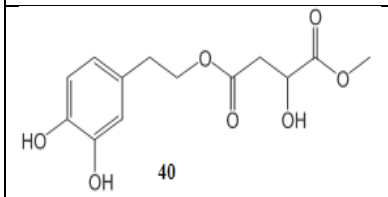
	**5ab:** Αλδεϋδική μορφή του άγλυκου συστατικού της ελαιοευρωπαΐνης (δυο στερεοισομερή) **6a,b:** Αλδεϋδική μορφή του άγλυκου συστατικού του λιγκστροζίτη (δυο στερεοισομερή Σημ: Έχει αναφερθεί σπανιότερα και τρίτο στερεοϊσομερές.	**6:** λιγκστράλη	**5**: Aldehydic form of oleuropein aglycon **6:** Aldehydic form of ligstroside aglycon/ ligstral	**5:** 3,4-DHPEA-EA **6:** p-HPEA-EA
	**7:** - **8:**- **Σημ: λόγω υδατοδιαλυτότητας δεν αναμένεται να υπάρχουν στο λάδι σε σημαντικές ποσότητες**	**7:** Ελαιοευρωπαΐνη **8:** Λιγκστροζίτης	**7:** Oleuropein **8:** Ligstroside	

ΠΑΡΑΡΤΗΜΑ Ι

	**9:** Άγλυκο ελαιοευρωπαΐνης **10:** Άγλυκο λιγκστροζίτη		**9:** Oleuropein aglycon **10:** Ligstroside aglycon	
	**11**: Διαλδεϋδική, μορφή του άγλυκου της ελαιοευρωπαΐνης (5S,4R και 5S,4S) **12:** Διαλδεϋδική μορφή του άγλυκου συστατικού του λιγκστροζίτη (5S,4R και 5S,4S)	**11**: ελαιοευρωπαϊνοδιάλη **12**: λιγκστροδιάλη	**11**: Dialdehydic form of oleuropein aglycon/ oleuropeindial **12:** Dialdehydic form of ligstroside aglycon/ligstrodial	
	**13:** ενολικό ταυτομερές της διαλδεϋδικής μορφής του άγλυκου της ελαιοευρωπαΐνης **14:** ενολικό ταυτομερές της διαλδεϋδικής μορφήςτου άγλυκου του λιγκστροζίτη	**13:** Ελαιομισσιονάλη **14:** Ελαιοκορωνάλη	**13:** enolic tautomer of the Dialdehydic form of oleuropein aglycon **14:** enolic tautomer of the Dialdehydic form of ligstroside aglycon	

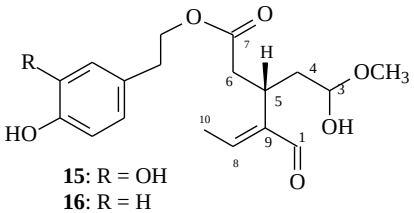
ΠΑΡΑΡΤΗΜΑ Ι

 **25**: R = OH **26**: R = H	**25:** Αποκαρβοξυμεθυλιωμένη μορφή του άγλυκου της ελαιοευρωπαΐνης **26:** Αποκαρβοξυμεθυλιωμένη μορφή του άγλυκου του λιγκστροζίτη **Σημ: Ασταθείς δομές που δεν είναι πιθανό να υπάρχουν υπο αυτή τη μορφή σε σημαντικές ποσότητες στο λάδι**		**25:** Decarboxymethyl form of oleuropein aglycon **26:** Decarboxymethyl form of ligstroside aglycon	
 **29** R=OH **30** R=H	**29:** Οξικός εστέρας της υδροξυτυροσόλης **30:** Οξικός εστέρας τυροσόλης		**29:** Hydroxytyrosol acetate **30:** Tyrosol acetate	
 **31** R=4-β-D-glucose	**31:** 4-β-D-γλυκοζίτης της υδροξυτυροσόλης **Σημ: λόγω υδατοδιαλυτότητας δεν αναμένεται να υπάρχει στο λάδι σε σημαντικές ποσότητες**		**31:** 4-β-D-glucoside of hydroxytyrosol	
 **32** R_1,R_2-H **33** R_1-OH,R_2-OCH_3	**32:** 1-φαινυλο-6,7-διυδροξυ ισοχρωμάνη **33:** 1-(3'-μεθοξυ-4'-υδροξυ) φαινυλο-6,7-διυδροξυ ισοχρωμάνη **Σημ: Είναι υπο συζήτηση αν θα πρέπει να περιληφθούν**		**32:** 1-Phenyl-6,7-dihydroxyisochroman **33:** 1-(3'-Methoxy-4'-hydroxy)phenyl-6,7-dihydroxyisochroman	

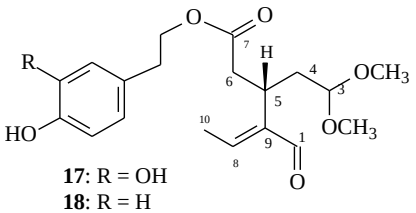
	**40:** εστέρας υδροξυτυροσόλης με το μεθυλο-μηλικό οξύ		**40:** β-hydroxytyrosol ester of methyl malate	

ΑΚΟΛΟΥΘΕΙ ΜΙΑ ΣΕΙΡΑ ΑΠΟ ΔΟΜΕΣ ΠΟΥ ΕΧΟΥΝ ΑΝΑΦΕΡΘΕΙ ΩΣ ΣΥΣΤΑΤΙΚΑ ΤΟΥ ΕΛΑΙΟΛΑΔΟΥ (Συνοπτικά αναφέρονται στο JAFC 2005,53,4331-4340) ΑΛΛΑ ΠΡΟΚΕΙΤΑΙ ΓΙΑ ΤΕΧΝΗΤΑ ΠΡΟΙΟΝΤΑ (artifacts)ΠΟΥ ΠΡΟΚΥΠΤΟΥΝ ΑΠΟ ΤΗΝ ΑΝΤΙΔΡΑΣΗ ΜΕ ΤΟΥΣ ΔΙΑΛΥΤΕΣ ΤΗΣ ΧΡΩΜΑΤΟΓΡΑΦΙΑΣ Ή ΓΙΑ ΥΠΟΘΕΤΙΚΈΣ ΔΟΜΕΣ ΠΟΥ ΔΕΝ ΕΧΟΥΝ ΠΕΡΙΓΡΑΦΕΙ ΜΕ ΠΛΗΡΗ ΦΑΣΜΑΤΟΣΚΟΠΙΚΑ ΣΤΟΙΧΕΙΑ

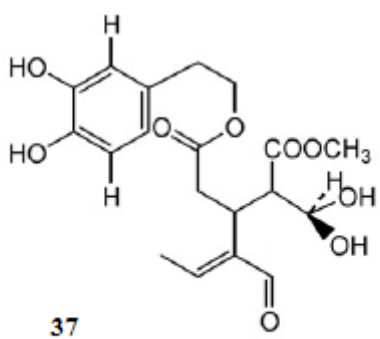
ΠΑΡΑΡΤΗΜΑ Ι



15: R = OH
16: R = H

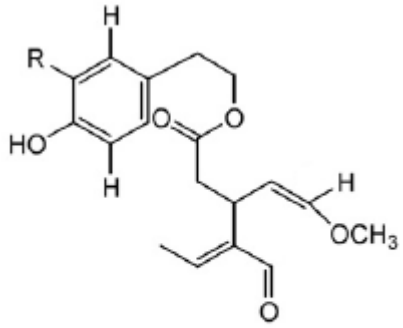


17: R = OH
18: R = H

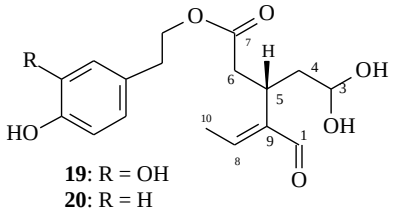


37

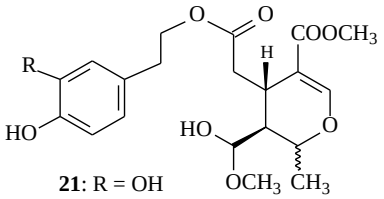
hydrated dialdehydic form of oleuropein



38 R = OH
39 R = H

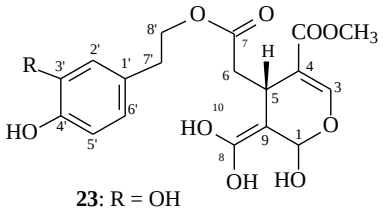


19: R = OH
20: R = H

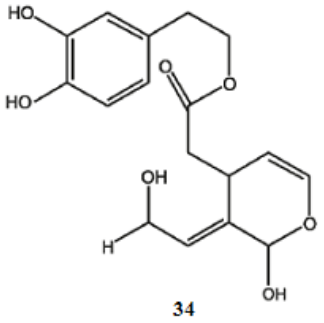


21: R = OH

22: R = H

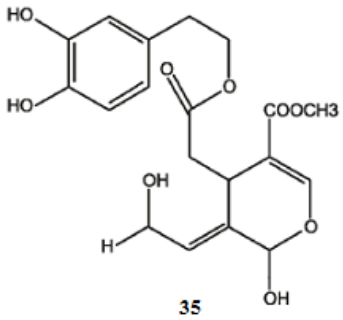


23: R = OH
24: R = H



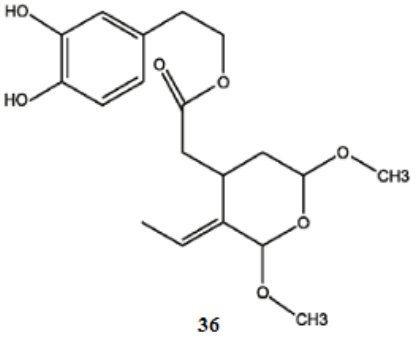
34

hydroxy-o-oleuropein aglycone lacking a carboxymethyl



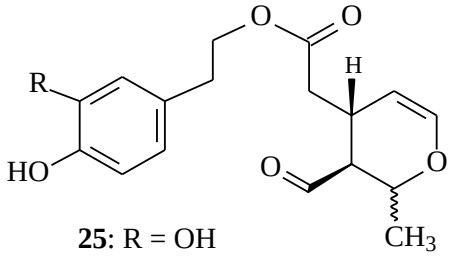
35

10-hydroxy-oleuropein aglycone



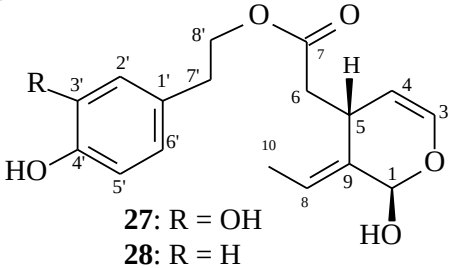
36

3,4-dihydroxyphenylethyl-(2,6-dimethoxy-3-ethylidene)tetrahydropyran-4-yl]acetate



25: R = OH

26: R = H



27: R = OH
28: R = H

ΣΗΜΕΙΩΣΗ:

Η κατάσταση των παραγώγων της τυροσόλης και υδροξυτυροσόλης πρέπει να ενημερώνεται κάθε φορά που ταυτοποιείται και νέα ένωση που σχετίζεται με τον ισχυρισμό υγείας του καν 432/12

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ΠΑΡΑΡΤΗΜΑ ΙΙ

Κατάλογος μεθόδων που έχουν δημοσιευθεί και μπορούν να χρησιμοποιηθούν για τον προσδιορισμό των ουσιών που αναφέρονται στον κανονισμό 432/2012 για τον ισχυρισμό υγείας για τις βιοφαινόλες ελαιολάδου.			
ΤΙΤΛΟΣ	ΣΥΓΓΡΑΦΕΙΣ	ΠΡΟΕΤΟΙΜΑΣΙΑ ΔΕΙΓΜΑΤΟΣ	ΜΕΘΟΔΟΣ ΑΝΑΛΥΣΗΣ
Determination of biophenols in olive oils by HPLC	International Olive Council COI/T.20/Doc No 29 November 2009	Direct extraction of the biophenolic minor polar compounds by means of methanol/water 80/20 (V/V) extraction solution	❖ Quantification by HPLC – DAD at 280nm with the aid of a UV detector at 280 nm ❖ Internal standard is used and specifically syringic acid The content of polyphenols is expressed in mg/kg of tyrosol
Analysis of Total Contents of Hydroxytyrosol and Tyrosol in Olive Oils	Concepción Romero and Manuel Brenes J. Agric. Food Chem. 2012, 60, 9017−9022	Olive oil (2.5−25 g) and 2 M HCl (25−50 mL) were put into a 100 mL glass bottle that was closed with a polypropylene cap. The mixture was vigorously homogenized by agitation at 400 rpm in an orbital shaking incubator	HPLC Analysis. Diode array and fluorescence detectors were used. Calibration curves were constructed for Htyr and Tyr
Characterization and Quantification of Phenolic Compounds in Olive Oils by Solid-Phase Extraction, HPLC-DAD and HPLC-MS/MS(*) (*)Η μέθοδος χρησιμοποιήθηκε σε μία από τις βασικές invivo μελέτες που υποστήριξαν επιστημονικά τον ισχυρισμό υγείας του καν(EU)432/2012.	Karina de la Torre-Carbot et al. J.Agric. Food Chem. 2005, 53, 4331-4340	The polar fraction was extracted using diol cartridges WatersVac RC 500mg - activation: 6m hexane, 6ml MeOH:H2O 8:2, 3ml MeCN) - percolation: 3g oil in 6ml hexane - washing: 10ml hexane - elution: 8ml MeOH:H2O 8:2, 4ml MeCN The entire process was performed in conditions of darkness and with brown glass material.	❖ Quantification by HPLC-DAD at 280, 240 and 320 nm ❖ Identification with HPLC-MS/MS ❖ Each phenolic compound is expressed with its standard when it is available: tyrosol, vanillic acid, vanillin, p-coumaric acid, oleuropein, luteolin, apigenin and methoxyluteolin - Secoiridoids are expressed as oleuropein - Tyr and HOTyr are expressed as Tyr - unknown flavonoids as luteolin The method is validated and precision values are given
Determination of phenol compounds in olive oils - Reference method	International Olive Council Chemists' meeting Madrid 2016	Direct extraction of the phenolic compounds from olive oil, either with MeOH/H2O 8/2 (LLE) or by SPE with diol	❖ Quantification by HPLC-DADat 280,335 nm ❖ Internal standard: - p-hydroxyphenyl acetic acid (280 nm)

		cartridges. In the LLE process, 4g oil are premixed with 1ml IS (p-OH-phenyl acetic acid and o-coumaric acid) and then extracted twice with 5ml MeOH/H2O 8/2 (vortex 1 min, ultrasounds 15 min). The combined extracts are centrifuged. The upper phase is analyzed with HPLC.	- o-coumaric acid (335 nm) Each phenolic compound is quantified using its response factor relative to the respective internal standard. Results in mg/kg. Relative response factors are given and are identical with those of Raquel Mateos et al., J.Agric. Food Chem. 2001, 49, 2185-2192
Direct measurement of oleocanthal and oleacein levels in olive oil by quantitative ^{1}H-NMR. Establishment of a new index for the characterization of extra virgin olive oils.	E. Karkoula, A. Skantzari, E. Melliou, P. Magiatis. J Agric Food Chem 60 (2012) 11696-11703. 10.1021/jf3032765	**Olive Oil Extraction and Sample Preparation for NMR Analysis.** Olive oil (5.0 g) was mixed with cyclohexane (20 mL) and acetonitrile (25 mL). The mixture was homogenized using a vortex mixer for 30 sec and centrifuged at 4,000 rpm for 5 min. A part of the acetonitrile phase (25 mL) was collected, mixed with 1.0 mL of a syringaldehyde solution (0.5 mg/mL) in acetonitrile and evaporated under reduced pressure using a rotary evaporator (Buchi, Switzerland).	**NMR Spectral Analysis**. The residue of the above procedure was dissolved in $CDCl_3$ (750 μL) and an accurately measured volume of the solution (550 μL) was transferred to a 5 mm NMR tube. ^{1}H-NMR spectra were recorded at 600MHz (Bruker Avance600) and 400 MHz (Bruker DRX400). Typically, 50 scans were collected into 32K data points over a spectral width of 0-16 ppm with a relaxation delay of 1 s and an acquisition time of 1.7 s. Prior to Fourier transformation (FT) an exponential weighing factor corresponding to a line broadening of 0.3 Hz was applied. The spectra were phased corrected and accurate integration was performed manually for the peaks of interest using TOPSPIN as described in details in references 1,2,3.
From Olive Drupes to Olive Oil. An HPLC-Orbitrap-based Qualitative and Quantitative Exploration of Olive Key Metabolites	Periklis Kanakis, Aikaterini Termentzi, Thomas Michel, Evagelos Gikas, Maria Halabalaki, Alexios-Leandros Skaltsounis Planta Med 2013; 79: 1576–1587	The polar fraction was extracted using diol cartridges. Briefly, after the activation of diol material with three column volumes of MeOH, the cartridges were conditioned with three column volumes of n-hexane (nHex). One hundred mg of each extract, diluted in nHex,were applied and eluted with five column volumes of the same	All analyses were performed on an Accela High Speed LC System equipped with a PDA detector and hyphenated to an LTQ-Orbitrap XL hybrid mass spectrometer, using an ESI ionisation probe, in the negative mode (Thermo Scientific). Calibration curves were constructed for nine standard compounds and Syringaldehyde was used as IS

		solvent. After the removal of the lipophilic compounds, the phenolics were eluted with five column volumes of MeOH. Finally, cartridges were washed with three column volumes of MeOH/H2O mixture (50/50). The recovery of all compounds quantified was estimated to be > 95%	
Evaluation of total hydroxytyrosol and tyrosol in extra virgin olive oils	Giorgia Purcaro, Rafael Codony, Lorena Pizzale, Carlo Mariani and Lanfranco Conte Eur. J. Lipid Sci. Technol. 2014, 116, 805–811	1) Polyphenol extraction procedure (following the International Olive Council method) 2) Hydrolysis procedure (by adding 1.5mL of the acetyl chloride solution to 1mL of a phenolic extract, previously completely evaporated, and let react at 80°C for 1h. 3) Derivatization step 4)	GC-FID analysis for quantification of tyrosol and hydroxytyrosol (Quantification was carried out by using the response factor (RF) method (with 1,3-OH-Tyr as IS internal standard)
Addressing Analytical Requirements To Support Health Claims on "Olive Oil Polyphenols" (EC Regulation 432/2012)	Aspasia Mastralexi , Nikolaos Nenadis, Maria Z. Tsimidou Agric. Food Chem, 2014, 62, 2459- 2461	1) Polar Fraction extraction The polar fraction was extracted from 2.5 g of VOO dissolved in 5 mL of hexane using an equal volume of methanol/water (60:40 v/v). 2) Acidic Hydrolysis according to Mulinacci et al. An aliquot (200 µL) from the polar fraction was mixed with 200 µL of a 1 M H 2SO4 solution. The mixture was maintained in a water bath at 80 °C for 2h	RP-HPLC Analysis. Diode array and fluorescence detectors were used in line. Calibration curves were constructed for Htyr and Tyr at appropriate wavelengths. Htyr and Tyr were quantified using respective external calibration curves.
Quantitative measurement of major secoiridoid derivatives in olive oil using qNMR. Proof of the artificial formation of aldehydic oleuropein and ligstroside aglycon	E. Karkoula, A. Skantzari, E. Melliou, P. Magiatis. J Agric Food Chem 62 (2014) 600-607. 10.1021/jf404421p	**Olive Oil Extraction and Sample Preparation for NMR Analysis.** Olive oil (5.0 g) was mixed with cyclohexane (20 mL) and acetonitrile (25 mL). The mixture was homogenized using a	**NMR Spectral Analysis**. The residue of the above procedure was dissolved in $CDCl_3$ (750 µL) and an accurately measured volume of the solution (550 µL) was transferred to a 5 mm NMR tube. ^{1}H-

isomers.		vortex mixer for 30 sec and centrifuged at 4,000 rpm for 5 min. A part of the acetonitrile phase (25 mL) was collected, mixed with 1.0 mL of a syringaldehyde solution (0.5 mg/mL) in acetonitrile and evaporated under reduced pressure using a rotary evaporator (Buchi, Switzerland).	NMR spectra were recorded at 600MHz (Bruker Avance600) and 400 MHz (Bruker DRX400). Typically, 50 scans were collected into 32K data points over a spectral width of 0-16 ppm with a relaxation delay of 1 s and an acquisition time of 1.7 s. Prior to Fourier transformation (FT) an exponential weighing factor corresponding to a line broadening of 0.3 Hz was applied. The spectra were phased corrected and accurate integration was performed manually for the peaks of interest using TOPSPIN as described in details in references 1,2,3.
Quantitative analysis of pungent and anti-inflammatory phenolic compounds in olive oil by capillary electrophoresis	Isabella Vulcano, Maria Halabalaki, Leandros Skaltsounis, Markus Ganzera Food Chemistry 169 (2015) 381–386	The samples were prepared by liquid–liquid partitioning following the protocol described by Rios-Martin and Gutierrez-Rosales (2010), with slight modifications. In brief, 5 mL of an ethanol/ water-mixture (8/2; v/v) was added to five gram olive oil, the mixture was vortexed for five min and then centrifuged. The polar extract (lower phase) was removed, the same extraction procedure repeated two more times and the combined solutions evaporated to dryness under reduced pressure. Prior to CE analysis they were dissolved in 5.00 mL anhydrous acetonitrile and washed two times with hexane to remove lipids and pigments. Finally, the ACN phase was membrane filtered and used for analysis.	Analytical experiments were performed on a 3D-CE system from Agilent (Waldbronn, Germany), equipped with autosampler, diode array detector (DAD) and temperature controlled column compartment. Separations were performed in fused-silica capillaries (50 lm i.d 52 cm effective length) purchased from Polymicro Technologies (Phoenix, AZ, USA). The developed CE-method was validated as required by ICH guidelines (International Conference on Harmonization guideline Q2(R1), 2005). Calibration curves were established by dissolving the reference compounds in non-aqueous acetonitrile and preparing individual concentration levels by serial dilution with the same solvent.

ΠΑΡΑΡΤΗΜΑ ΙΙ

Extraction, Separation, and Identification of Phenolic Compounds in Virgin Olive Oil by HPLC-DAD and HPLC-MS	Maria Tasioula-Margari and Eleftheria Tsabolatidou Antioxidants 2015, 4, 548-562; doi:10.3390/antiox4030548	Olive oil (5g) was extracted with 5ml MeOH (3times). The extracts were evaporated and the residue was diluted in 5ml acetonitrile. Two subsequent washes with hexane were performed.	HPLC analysis. Two detectors were used, DAD and MS.
Oleokoronal and oleomissional: new major phenolic ingredients of extra virgin olive oil"	Panagiotis Diamantakos, Angeliki Velkou, K Brian Killday, Thanasis Gimisis, Eleni Melliou, Prokopios Magiatis. **OLIVAE** 2015, 122, 22-35	**Olive Oil Extraction and Sample Preparation for NMR Analysis.** Olive oil (5.0 g) was mixed with cyclohexane (20 mL) and acetonitrile (25 mL). The mixture was homogenized using a vortex mixer for 30 sec and centrifuged at 4,000 rpm for 5 min. A part of the acetonitrile phase (25 mL) was collected, mixed with 1.0 mL of a syringaldehyde solution (0.5 mg/mL) in acetonitrile and evaporated under reduced pressure using a rotary evaporator (Buchi, Switzerland).	**NMR Spectral Analysis**. The residue of the above procedure was dissolved in $CDCl_3$ (750 μL) and an accurately measured volume of the solution (550 μL) was transferred to a 5 mm NMR tube. ^{1}H-NMR spectra were recorded at 600MHz (Bruker Avance600) and 400 MHz (Bruker DRX400). Typically, 50 scans were collected into 32K data points over a spectral width of 0-16 ppm with a relaxation delay of 1 s and an acquisition time of 1.7 s. Prior to Fourier transformation (FT) an exponential weighing factor corresponding to a line broadening of 0.3 Hz was applied. The spectra were phased corrected and accurate integration was performed manually for the peaks of interest using TOPSPIN as described in details in references 1,2,3.
A widely used spectrophotometric assay to quantify olive oil biophenols according to the health claim (EU Reg. 432/2012)	Patricia Reboredo-Rodr ıguez, Enrico Valli, Alessandra Bendini, Giuseppe Di Lecce, Jesus Simal-Gandara and Tullia Gallina Toschi Eur. J. Lipid Sci. Technol. 2016, 118, 0000–0000	1) Polyphenol extraction procedure (following the International Olive Council method with some modifications) 2) Acid hydrolysis was carried out by adding 1mL of 5M HCl to 1mL of hydroalcoholic phenolic extract and let react at 100°C in an oven for 1h 3) The Folin-Ciocalteu assay was performed.	Spectrophotometric analysis using an UV–Vis 1800 spectrophotometer. Phenolic compounds were detected at 750nm and quantified using HTyr calibration curve The data were expressed as mg HTyr $20g^{-1}$ of oil.

ΠΑΡΑΡΤΗΜΑ ΙΙ

Quantitative method for determination of oleocanthal and oleacein in virgin olive oils by liquid chromatography–tandem mass spectrometry	V. Sanchez de Medina, H. Miho, E. Melliou, P.Magiatis, F. Priego-Capote, M.D. Luque de Castro. Talanta 162 (2017) 24-31	Olive oil is extracted with hexane/Acetonitrile or hexane/MeOH:water 60:40	The acetonitrile or MeOH:water 60:40 extract is analyzed with LC-QqQ MS/MS using pure oleocanthal and oleacein for calibration