Latest International Olive Council Recommendations for Addressing Analytical Requirements to Support Health Claims on“Olive Oil Polyphenols” (EC Regulation 432/2012)

Maria Tsimidou

Professor emerita

Coordinator METRO FOOD·GR JOINT RESEARCH UNIT METROFOOD-GR

Lab. Food Chemistry and Technology, School of Chemistry, Aristotle University of Thessaloniki, Greece, 54124

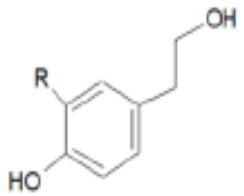
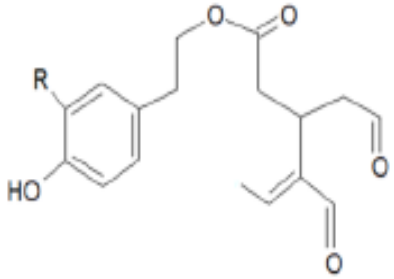
tsimidou@chem.auth.gr

Introduction

- Olive drupes contain an interesting gamut of minor compounds that are transferred to virgin olive oil (VOO) during processing by only physical means.

- Among them, **derivatives of the secoiridoids oleuropein & ligstroside** monopolized the interest of researchers, first, for technological reasons, as they were related to the remarkable oxidative stability of the oil, its taste and, later on, due to a variety of health benefits.

Table 1. Chemical structure, IUPAC/empirical nomenclature and common abbreviation of the most known oleuropein and ligstroside derivatives present in virgin olive oil

Chemical structure	IUPAc/empirical nomenclature	Common Abbreviation
	1: (3,4-dihydroxyphenyl) ethanol/ hydroxytyrosol, Hytyr	1: 3,4-DHPEA
	2: (*p* -hydroxyphenyl) ethanol/ tyrosol, Tyr	2: *p*-HPEA
	3: dialdehydic form of decarboxymethyl elenolic acid linked to 3,4-DHPEA/oleacein	3: 3,4-DHPEA-EDA
	4: dialdehydic form of decarboxymethyl elenolic acid linked to *p* –HPEA/oleocanthal	4: *p*-HPEA-EDA

1 and 3: R = OH; 2 and 4: R = H

Being more polar in nature, and in order to differentiate them from the less polar tocopherols, this group of compounds was named **olive oil 'polar phenols'**, or **olive oil 'polyphenols',**

These compounds are commonly extracted from the lipid matrix using **aqueous methanol solutions.** This extract is mentioned as olive oil **polar fraction (PF)**

The term **'polyphenols'** is still in use even in official documents, though the most abundant ones bear only one phenolic ring. **The term 'polar phenols' (PPh) is adopted subsequently for the aim of the presentation.**

The term 'olive oil polyphenols' will be used exclusively when it refers to the EC Regulation 432/2012 and the related European Food Safety Authority (EFSA) opinion.

One of the **latest analytical challenges** concerning olive oil is that raised after issuing of EU Reg. 432/2012, which authorizes a health claim substantiated by an EFSA scientific opinion [EFSA J. 2011, 9(4) 2033]

Claim type	Nutrient, substance, food or food category	Claim	Conditions of use of the claim / Restrictions of use / Reasons for non-authorisation
Art.13(1)	Olive oil polyphenols	Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress	The claim may be used only for olive oil which contains at least 5 mg of hydroxytyrosol and its derivatives (e.g. oleuropein complex and tyrosol) per 20 g of olive oil. In order to bear the claim information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 20 g of olive oil.

- lack of an official or recommended method that could be unanimously used for the determination of the target compounds

- lack of consensus among scientists and authorities regarding which individual compounds among the numerous known olive oil phenolics should be summed up to give min 5 mg/20 g oil

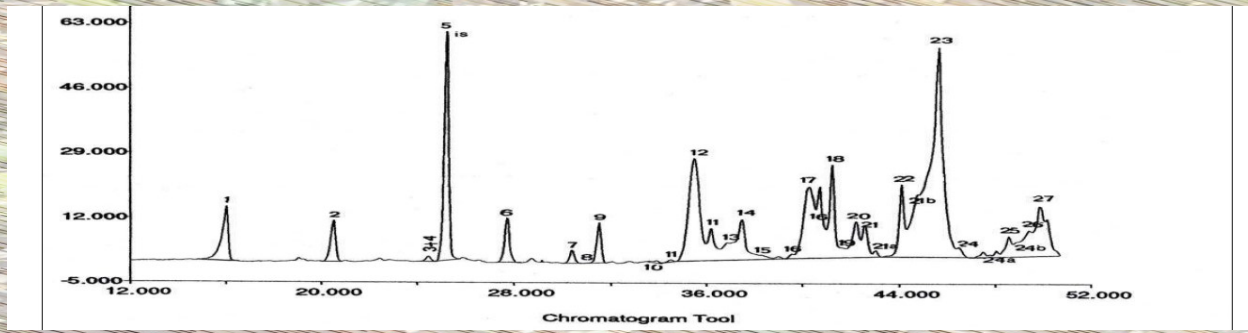
This challenge

Led olive oil sector to limit implementation of this claim to avoid legal implications

IOC acknowledged the inappropriateness of the COI/T.20/Doc No 29 method (Determination of biophenols in olive oils by HPLC) for such a purpose

IOC opened a consultation "on the modification of the method for polyphenol quantification"

http://www.internationaloliveoil.org/news/view/666-year-2014- news/483-new-procedure-for-presenting-proposals-for-the-revision-ofioc-standards



RPHPLC-UV

As a consequence, various separation protocols, were developed over the last decade to address gaps at all stages of polar phenol analysis

Table 2 shows the dedicated separation protocols for the implementation of the health claim on ‘olive oil polyphenols’ that were published from 2011 to June 2022, when the last version of the IOC document, no. 29 , was uploaded to its official website.

Other approaches have been proposed, too

Table 2: Dedicated separation protocols to support PPh health claim 2011-22

Reference/Publication Year	Research Unit
Romero and Brenes [2]/2012	Instituto de la Graca/Sevilla/ES
Mastralexi et al. [2]/2014	Aristotle University of Thessaloniki/GR
Purcaro et al. /2014	University of Udine/IT; University of Barcelona/ES; Stazione Sperimentale per le Industrie degli Oli e dei Grassi/Milano/IT
Tasioula-Margari and Tsabolatidou /2015	University of Ioannina/GR
Monasterio et al. 2016	Instituto de Biología Agrícola de Mendoza/AR; University of Granada/ES
Ricciutelli et al. /2016	University of Camerino/IT
Bartella et al. /2018	Università della Calabria/Rende/IT
Celano et al. /2018	University of Salerno, Fisciano, IT; University of Reggio Calabria, IT
Nenadis et al. /2018	Aristotle University of Thessaloniki, GR; University of Perugia/IT; Instituto de la Graca/Sevilla/ES; Alma Mater Studiorum/University of Bologna/IT
Olmo-García et al. /2019	University of Granada/ES; CM Europa S.L/Martos/ES
Ferro et al. /2019	Instituto Politécnico de Beja/PT; University of Aveiro/PT; Universidade de Évora/PT
Tsimidou et al. /2019	Aristotle University of Thessaloniki/GR; Instituto de la Grasa (CSIC)/Seville/ES, Alma Mater Studiorum-University of Bologna/IT
Tsimidou et al. /2019	Aristotle University of Thessaloniki/GR; University of Perugia/IT; Institute for Oliveculture, Science and Research Centre Koper, Zagreb/SI; Universitat de Barcelona/ES; Eurofins Analytik GmbH/ Hamburg/DE; Instituto de la Grasa (CSIC)/Seville/ES; Alma Mater Studiorum—Univ. of Bologna/IT
Bellumori et al. /2019	University of Firenze and Multidisciplinary Centre of Research on Food Sciences (M.C.R.F.S.-Ce.R.A.)/IT; University of Bari/IT University Aldo Moro/Bari/IT
Pereira et al. /2020	Universidade de Évora/PT
Termopoli et al. /2021	University of Urbino/IT; Vancouver Island University/Nanaimo/CA
Paradiso et al. /2022	University of Salento/Lecce/IT; University of Bari, Italy/; University of Firenze/IT

Until 2017, the IOC had not made any proposal despite the pressure from the national authorities and the olive oil industry.

International Olive Council [IOC]. *Determination of Biophenols in Olive Oil by HPLC. COI/T.20/Doc. No. 29 Rev.1*; International Olive Council [IOC]: Madrid, Spain, 2017. Available online: https://www.internationaloliveoil.org/wp-content/uploads/2019/11/COI-T.20-Doc.-No-29-Rev-1-2017.pdf

The method is based on direct extraction of the biophenolic minor polar compounds from olive oil by means of a methanol solution and subsequent quantification by HPLC with the aid of a UV detector at 280 nm. Syringic acid is used as the internal standard.

The content of the natural and oxidised oleuropein and ligstroside derivatives, lignans, flavonoids and phenolic acids is expressed in mg/kg of tyrosol.

2009

The method is based on direct extraction of the biophenolic minor polar compounds from olive oil by means of a methanol solution and subsequent quantification by HPLC with the aid of a UV detector at 280 nm. Syringic acid is used as the internal standard.

The content of the natural and oxidised oleuropein and ligstroside derivatives, lignans, flavonoids and phenolic acids is expressed in mg/kg of tyrosol.

2017, no mention to health claim

In June 2022,

the IOC concluded its procedures to address the analytical issue.

Thus,

in the last version of Doc No 29, two methods are proposed:

Method 1, COI/T.20/Doc. No 29/Rev.1 2017, for the determination of the biophenols in olive oils using HPLC,

&

Method 2, for the determination of phenolic compounds in olive oils using SPE-HPLC-DAD.

INTERNATIONAL
OLIVE
COUNCIL

COI/T.20/Doc. No 29/Rev.2
June 2022

ENGLISH
Original: ITALIAN

Príncipe de Vergara, 154 – 28002 Madrid – España Telef.: +34 915 903 638 Fax: +34 915 631 263 - e-mail: iooc@internationaloliveoil.org - http://www.internationaloliveoil.org/

DOCUMENT TO DECLARE THE USE OF IOC METHODS FOR PHENOLIC COMPOUNDS DETERMINATION

METHOD 1: COI/T.20/Doc. No 29/Rev.1 2017. DETERMINATION OF BIOPHENOLS IN OLIVE OILS BY HPLC

The method is based on the direct extraction of the BMP compounds from olive oil by means of a methanol solution and subsequent quantification by HPLC with the aid of a UV detector at 280 nm. Syringic acid is used as the internal standard.

The content of the natural and oxidised oleuropein and ligustroside derivatives, lignans, flavonoids and phenolic acids is expressed in mg/kg of tyrosol.

METHOD 2: DETERMINATION OF PHENOLIC COMPOUNDS IN OLIVE OILS BY SPE-HPLC-DAD

The method is based on the direct extraction of the phenolic compounds from olive oil by SPE on diol-phase cartridges and subsequent quantification directly by HPLC-DAD.

Differences

Both methods isolate the phenolic fraction from olive oils by different means: liquid-liquid extraction vs SPE extraction. In collaborative tests performed within the IOC, differences were observed when comparing the results obtained by the different methods due to the saturation of the liquid phase as the concentration of phenol increases (method COI/T.20/Doc. No 29 includes a range of determination, whereas the new method does not). The use of only an internal standard may underestimate flavonoids and ligustrosides. The new method also uses response factors for the different phenolic compounds whereas method No 29 quantifies using tyrosol and therefore may underestimate the true value.

USES

Method COI/T.20/Doc. No 29 may be used to perform a quick determination of phenols using a simpler method, whereas the new method can be used to determine the real concentration of phenols to fulfil an EFSA claim as well as the content of individual phenols (e.g., oleocanthal and oleoscein).

Which is the new method?
Who developed it? When and for which purpose?
Is it validated to fit the purpose?

Table 3 Who and when ??

SPE on Diol Cartridge by Mateos et al., 2001	SPE on Diol Cartridge using IOC Method no. 29(2) 2022
❖ A diol-bonded phase cartridge was placed in a vacuum elution apparatus and conditioned by the consecutive passing of 6 mL of methanol and 6 mL of hexane. ❖ The vacuum was then released to prevent drying of the column. ❖ The oil solution was applied to the column, and the solvent was pulled through, leaving the sample and the standard in the solid phase . ❖ The sample container was washed with two 3 mL portions of hexane, which were run out of the cartridge. ❖ The sample container was washed again with 4 mL of hexane/ethyl acetate (90:10, v/v), which was run out of the cartridge and discarded. ❖ Finally, the column was eluted with 10 mL of methanol, and the solvent was evaporated in a rotary evaporator at room temperature under vacuum until dry. ❖ The residue was extracted with 500 µL of methanol/water (1:1, v/v) at 40 °C. ❖ An aliquot (20 µL) of the final colorless solution was injected into the HPLC system.	❖ Place the SPE diol cartridge in the SPE equipment. Activate the cartridge by passing 6 mL of methanol and 6 mL of n-hexane without vacuum. ❖ Make sure the cartridge does not dry during elution. ❖ The oil sample is diluted with 6 mL of n-hexane and placed into the activated SPE cartridge. Let the sample enter the cartridge. ❖ Wash the flask with 6 mL of n-hexane and place into the column. Leave it to run out of the cartridge and discard. ❖ Elute with 4 mL of the eluting mixture n-hexane:ethyl acetate (85:15, v/v) and discard. ❖ Elute with 10 mL of methanol and collect the elution in a 25 mL conic flask. Evaporate in a rotary evaporator at room temperature in a vacuum until dry. ❖ Dissolve the residue in 500 µL of the methanol/water mixture then shake it vigorously with the aid of a vortex. ❖ Keep this final solution in dark and cool conditions for at least four hours before determination.

Table 4. HPLC conditions proposed for 'olive oil polyphenols' according to:

	Mateos et al., 2001	IOC Method no. 29(2) 2022
Column	Lichrospher 100RP-18 column (4.0 mm i.d. × 250 mm; particle size, 5 µm) maintained at 30 °C.	RP18 (4.0 mm i.d. × 250 mm length), 5 µm
Eluting solvents	A, water/acetic acid (97:3, v/v)	A, water:phosphoric acid (99.5:0.5, v/v).
	B, methanol/acetonitrile (50:50 v/v)	B, methanol:acetonitrile (1:1, v/v)
Gradient	5% B (0 min); 30% B (25 min); 35% B (35 min); 40% B (40min); 70% B (50 min) and 100% B (55 min), followed by 5 min of maintenance.	5% B (0 min); 30% B (15 min); 38% B (30 min); 45% B (40 min); 52.5%B (45 min); 100%B (50 min)
Flow rate	1.0 mL/min	1 mL/min
Quantification	Internal standard solution, p-hydroxyphenylacetic acid, 4.64×10^{-2} mg/mL and o-coumaric acid, 9.6×10^{-3} mg/mL in methanol	Internal standard solution, p-hydroxyphenylacetic acid, 0.12 mg/mL and o-coumaric acid, 0.01 mg/mL in methanol.

Mateos, R.; Espartero, J.L.; Trujillo, M.;. Ríos, J.J.; León-Camacho, M.; Alcudia, F.; Cert, A. Determination of Phenols, Flavones, and Lignans in Virgin Olive Oils by Solid-Phase Extraction and High-Performance Liquid Chromatography with Diode Array Ultraviolet Detection. *J. Agric. Food Chem.* **2001**, *49*, 2185−2192. https://doi.org/10.1021/jf0013205

A simple analytical method for the quantitative determination of phenols, flavones, and lignans in virgin olive oils was developed. The polar fraction was isolated from small amounts of oil sample (2.5 g) by solid-phase extraction (SPE) using diol-phase cartridges, and the extract was analyzed by reversed-phase HPLC coupled with diode array UV detection. Chromatographic separation of pinoresinol, cinnamic acid, and 1-acetoxypinoresinol was achieved. Repeatability (RSD < 6.5%), recovery (> 90%), and response factors for each identified component were determined. SPE on amino-phase cartridges was used for isolating acidic phenols and as an aid for phenol identification. For the first time, 2-(4-hydroxyphenyl)ethyl acetate was detected in olive oils. The aldehydic structure of the ligstroside aglycon was confirmed by NMR spectroscopy. The colorimetric determination of total *o*-diphenolic compounds by reaction with molybdate was consistent with their HPLC determination. **Differences between results obtained by liquid-liquid extraction and SPE were not statistically significant.**

A detailed analytical work obviously not dedicated to EFSA claim!

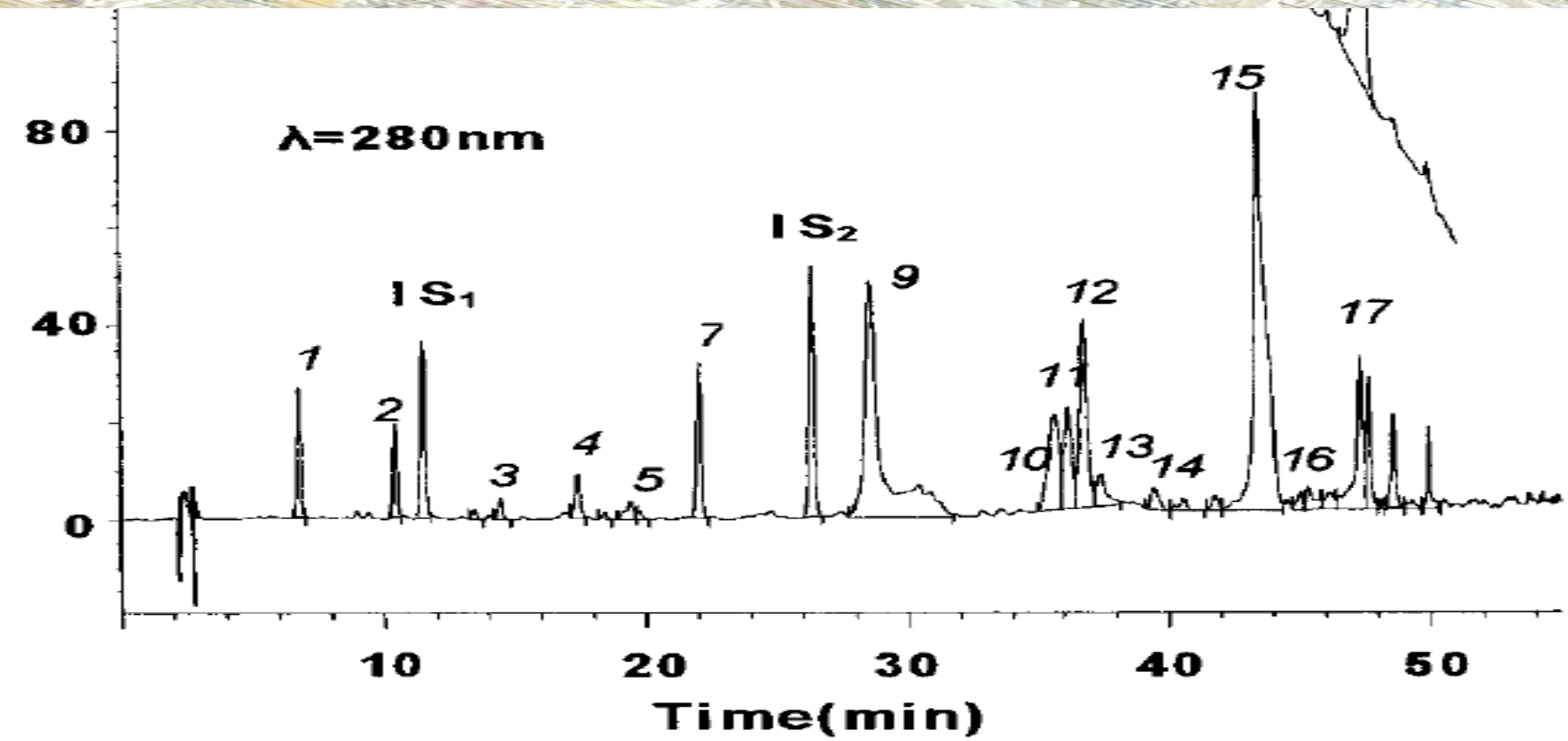


Figure 2. HPLC chromatogram of phenolic compounds isolated from Picual virgin olive oil by SPE on diol phase (detection at $\lambda = 280$, 240, and 335 nm). Peaks: (1) hydroxytyrosol; (2) tyrosol; (IS_1) *p*-hydroxyphenylacetic acid; (3) vanillic acid; (4) vanillin; (5) *p*-coumaric acid; (7) hydroxytyrosyl acetate; (8) elenolic acid; (IS_2) *o*-coumaric acid; (9) dialdehydic form of decarboxymethyl oleuropein aglycon; (10) dialdehydic form of decarboxymethyl ligstroside aglycon; (11) pinoresinol; (12) cinnamic acid; (13) 1-acetoxypinoresinol; (14) luteolin; (15) aldehydic form of oleuropein aglycon; (16) apigenin; (17) aldehydic form of ligstroside aglycon.

ISO Method 2 is the result of collaborative tests performed by 20 laboratories within the IOC.

Precision data from the interlaboratory study organized by the IOC are given for the total phenol content (mg/kg) after summing up 15 compounds, among which are vanillic acid, vanillin, ferulic acid, pinoresinol, cinnamic acid, 1-acetoxypinoresinol, luteolin and apigenin.

Those eight compounds in particular are not secoiridoids and are not considered in the 5 mg of hydroxytyrosol and its derivatives (e.g., oleuropein complex and tyrosol) per 20 g of olive oil required by the health claim.

The IOC recommendation ignores the endeavors of so many scientists to address the analytical gaps in specific and unambiguous ways over the last decade.

The proposed analytical protocol for extraction, separation, identification and quantification seems to be an outdated compromise,

whereas

there are more recent validated protocols ‘fit for the purpose’ of the EFSA health claim available

Conclusions

- Unequivocally, authorization of the ‘olive oil polyphenol’ health claim boosted PPh analysis of virgin olive oils from different cultivars and countries, which was a positive activity for the overall interests of SMEs and small producers.

- Even though the claim could not be used on the labeling for 10 years already, publicity and consumer awareness of the health benefits of olive oil polyphenols was gained in various ways, mainly through social media and scientific publications.

- However, the bureaucratic procedures of international bodies do not seem to follow scientific progress for the benefit of the olive sector.

- Researchers from more than 26 institutes who worked hard in this direction should be rather disappointed.

- **The analytical gap cannot be considered addressed on the soundest scientific basis by the recently recommended method of the IOC.**

Maria Z. Tsimidou

Separations **2022**, *9*, 351. https://doi.org/10.3390/separations9110351

IOC

HUILES D'OLIVE - OLIVE OILS

Tableau 1 : PRODUCTION (1.000 tm) - Table 1: PRODUCTION (1,000 tonnes)

production - consumption

	2020/21 (31)	2021/22 (32)	2022/23 (prov.) (33)	2023/24 (prev.) (34)	2020/21 (31)	2021/22 (32)	2022/23 (prov.) (33)	2023/24 (prev.) (34)
Chypre / Cyprus	4,5	4,0	4,8	5,1				
Croatie / Croatia	3,7	2,9	5,1	2,5	5,3	5,4	4,9	6,5
Espagne / Spain	1.389,0	1.491,5	665,8	766,4	7,6	8,5	8,2	8,0
France / France	4,5	5,8	3,6	3,6	541,1	587,0	359,8	350,0
Grèce / Greece	275,0	232,0	345,0	195,0	139,9	141,7	104,3	95,0
Italie / Italy	273,5	329,0	240,9	268,9	108,6	105,5	97,6	80,0
Malte / Malta	0,0	0,0	0,0	0,0	421,9	453,6	478,8	415,0
Portugal / Portugal	100,0	206,2	126,0	150,0	1,1	1,7	0,9	1,0
Slovénie / Slovenia	0,9	0,3	0,7	0,5	61,8	59,0	47,5	50,0
TOTAL A)	2.051,2	2.271,7	1.391,9	1.413,0	2,8	2,2	2,3	2,5
					1.290,1	1.364,6	1.104,3	1.008,0
Allemagne 1) / Germany 1)					82,0	74,2	49,3	74,5
Autriche / Austria					8,7	10,3	7,2	7,2
Belgique / Belgium					11,1	13,7	13,0	13,2
Bulgarie / Bulgaria					5,3	6,0	1,9	2,5
Danemark / Denmark					6,0	5,6	4,2	4,1
Estonie / Estonia					0,8	0,7	0,5	0,9
Finlande / Finland					2,9	2,9	2,2	2,7
Hongrie / Hungary					3,3	3,3	2,3	2,5
Irlande / Ireland					3,7	3,7	3,7	3,9
Lettonie / Latvia					0,7	1,0	0,5	1,3
Lituanie / Lithuania					1,2	1,1	0,6	2,1
Luxembourg / Luxembourg					1,6	1,7	1,3	2,8
Pays-Bas / Netherlands					21,0	17,9	12,1	13,2
Pologne / Poland					11,2	17,2	11,4	10,0
Rép.Tchèque / Czech. Rep.					5,0	4,3	3,3	5,5
Roumanie / Romania					5,9	4,9	4,0	3,5
Royaume-Uni / United King.					-	-	-	-
Slovaquie / Slovakia					2,9	1,8	1,5	1,6
Suède / Sweden					13,2	12,3	8,8	10,5
TOTAL B)	0,0	0,0	0,0	0,0	186,5	182,6	127,8	162,1
TOTAL A + B	2.051,2	2.271,7	1.391,9	1.413,0	1.476,6	1.547,2	1.232,1	1.170,1

Climate change… lower production… higher prices… lower consumption

NEWS ARTICLES 27 February 2024: **Price of olive oil up 50% in one year**
https://ec.europa.eu/eurostat/web/products-eurostat-news/w/ddn-20240227-1

Olive oil fraud and mislabelling cases hit record high in EU

Fifty potential cases in first three months of this year as rising costs lead to increase in parallel market

https://www.theguardian.com/world/article/2024/jul/29/olive-oil-fraud-mislabelling-cases-record-high-eu

[Spanish Households Purchase More Sunflower Oil Than Olive Oil for the First Time]

Falling olive oil sales are linked to reduced production and elevated prices, whereas sunflower oil has become more affordable during the same timeframe.

https://www.oliveoiltimes.com/business/europe/record-olive-oil-prices-drive-food-inflation-in-greece/132220

"Greek consumers have reduced their consumption of olive oil by up to 40 percent due to the high prices and have turned to other oils, such as seed oil," said Manolis Giannoulis, the head of EDOE, the national interprofessional olive oil association.

Thank you for your attention

26th November “World Olive Day”

https://metrofood.gr/media-room/