Why Tyrosol Derivatives Have to Be Quantified in the Calculation of “Olive Oil Polyphenols” Content to Support the Health Claim Provisioned in the EC Reg. 432/2012

Viewpoint – Pre-print version

Why tyrosol derivatives have to be quantified in the calculation of "olive oil polyphenols" content to support the health claim provisioned in the EC Reg. 432/2012

Running title: Tyrosol and derivatives and the "olive oil polyphenol" health claim

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Abstract: The viewpoint is the outcome of the scientific expertise of the scientists that sign it and work collaboratively in the frame of the OLEUM project. The project aims to better guarantee olive oil quality and authenticity by empowering detection and fostering prevention of olive oil fraud and by an effort of harmonization, correct interpretation and use of official and supporting analytical methods.

Practical applications: The consensus among scientists, the European food authorities, IOC and the olive industry on which compounds should be determined to support the health claim on olive oil polyphenols (EC Reg. 432/2012) is of utmost importance and can be supported by the evidence provided in this viewpoint article.

Keywords: Hydroxytyrosol/ Tyrosol/ "olive oil polyphenols" / Health claim

1. The issue

The health claim on the phenolic compounds of olive oil of the EC Regulation 432/2012 is spelled as shown in Figure 1.[1] It is based on the relevant EFSA (European Food Safety Authority) scientific opinion[2] and adopts the terminology introduced in the latter. The wording "hydroxytyrosol and derivatives" accompanied by an explanation in parenthesis "(e.g. oleuropein complex and tyrosol)" being not further detailed in the EFSA publication triggered several discussions among interested parties regarding its unequivocal interpretation.[3] As a result, there is a need of clarification about which compounds should be summed up to give the amount of at least 5 mg phenols/20 g oil and the benefits of using such a claim for commercial reasons are still not enough explored by stakeholders. Nevertheless, almost at the same period different analytical approaches appear in literature to address this issue.[4-9]

2. The opinion

In the olive drupe hydroxytyrosol (Htyr) and tyrosol (Tyr) are biosynthetically interrelated as is illustrated in Figure 2.[10] As stated by those authors "a strong correlation was observed between phenolic compound concentrations and transcripts putatively involved in their biosynthesis, suggesting a transcriptional regulation of the corresponding pathways" for the two studied olive varieties. Consequently, Tyr and its derivatives may be converted to Htyr and derivatives and vice versa in the drupe. The extent of conversion, which will be reflected in their concentration in olive oil, depends on the cultivar, fruit ripening, climate conditions, soil, water availability and agricultural practices.[11] Upon processing these compounds in the same or further modified structure are determined in virgin olive oil, which, when freshly extracted from healthy olives of the appropriate maturity index, contains mainly bound forms (Table 1 and 2). In this view the wording[1] also implies the presence of Tyr and derivatives. The possible uncertainty comes from the information given in parenthesis as an example, i.e."(e.g. oleuropein complex and tyrosol)" in [1] and the fact that, in the respective EFSA scientific opinion paper,[2] three different expressions are used irrespectively, in different parts of the text, to describe the health claim: (i) "hydroxytyrosol and derivatives" without any further explanation in parenthesis, (ii) "(e.g. oleuropein complex and tyrosol)" as an explanation to (i) and (iii) (e.g. oleuropein complex)"as an explanation to (i).

There is no doubt that the expression "oleuropein complex" should include all of the compounds that bear the hydroxytyrosol moiety and have been identified in virgin olive oil so far using different techniques.[12-18] These compounds are shown in Table 1. In the same table the peak number, corresponding to the elution order according to the IOC (International Olive Council) HPLC protocol, is given, where available.[19] Compounds such as *β*-hydroxytyrosol ester of methyl malate that has been reported only in the drupe and allegedly may pass in the oil,[20] oleuropein that is rarely reported in the fresh virgin olive, as it is hydrolysed during processing, as well as 10-hydroxy-oleuropein,[15] hydroxyl-isochromans, which have been identified but at negligible levels (less than 1.4 ppb and down to ppt), requiring, thus, MS/MS for their quantification,[21] and hydroxytyrosol glucosides[22] expected to be low (<5 ppm) or even not detected with MS,[23] are not included in this table. In this sense, the expression "oleuropein complex" should include all the compounds listed in Table 1 in their free or bound form, in line with the fact that both free and bound forms of

Htyr are present in olive oil. However, the term "tyrosol" needs to be further and definitively clarified, because it could be incorrectly interpreted as referred only to the free form. This point raises doubts whether the EFSA panel indented to include only the free form of this monophenol in the calculation of the minimum amount of 5 mg phenols/20 g oil and needs to be clarified for a harmonized interpretation and correct calculation of the compounds that should be summed in a future dedicated and standardized method. Tyrosol, written in parenthesis, is given as an example and it can be deduced that tyrosol derivatives (Table 2) should be also summed up. Vissers et al.[24] working out the olive oil phenol intake as 9 mg/day in the Mediterranean countries, estimated that 1 mg is derived from free Htyr and Tyr and 8 mg from their aglycones". In a recent review, Covas et al.[25], the group that carried out the research[26] on which EFSA opinion relied to set the quantitative limit for the health claim, clearly takes into consideration Tyr and derivatives in the calculation of the 5 mg/20g of olive oil.

3. Documentation of why tyrosol derivatives should be considered in the calculation of "olive oil polyphenols" content

Even if the statement appears enough clear so as not to require any further official clarification by EFSA, this is a point that needs scientific justification and consensus among all the interested parties. For this reason, we further document here why tyrosol derivatives should be also summed up in the calculation of the mg of the bioactive phenols that contribute to the protection of blood lipids from oxidative stress. Such a clarification is a red line to further address analytical aspects of the methodology that is most appropriate for the determination of the responsible compounds and the standards that should be used for their accurate quantification. The amount required by the health claim will be substantially influenced if these derivatives will or will not be summed up. This view is supported by data shown in Table 3,[4-6, 27-32] which prove that Tyr and derivatives are found to similar quantities as those of the oleuropein complex.

Documentation is provided in review articles and book chapters[25, 33-35] and additional publications.[36-42] In brief, at dietary doses of olive oil, Tyr and its derivatives are absorbed by humans. The complex forms are expected to be hydrolyzed in the gastrointestinal (GI) track giving rise to Tyr, which is absorbed in the small intestine. The latter is the major site of absorption. The hydrolysis of Tyr complex forms in the GI track is incomplete but degradation may also occur in the large intestine by colonic microflora liberating free Tyr, which is then absorbed. The complex forms of ligstroside aglycone and deacetoxyaglycone are absorbed and metabolized since their hydrogenated and/or glucuronated derivatives have been detected in human urine after 2h of olive oil intake. Tyr is present in the form of glucuronide derivative in plasma and in this form is bound to low density lipoprotein (LDL). This is the main form also in urine because the free form detected accounted for the 11-13% of the total recovered Tyr content, suggesting absorption and first pass intestinal/hepatic metabolic conversion. Recent studies in Wistar rats and human liver microsomes and baculosomes showed that Tyr is also converted to Htyr *in vivo*. The dietary pattern may affect positively or negatively the bioavailability of Tyr. Maximum excretion of dietary Tyr in urine has been reported after 6 h.

Regarding substantiation of the contribution of Tyr and derivatives to the protection of blood lipids from oxidation there is no available information from *in vivo* studies. This is due to the fact that investigations carried out in humans or animals comment on the effect observed as a function of the total phenol dose administered. Thus, the evidence presented is that a high intake of polar phenols results in an increase of phenols in plasma. Such an increase correlates with the decrease of oxidized LDL (oxLDL) or other lipid oxidation indices, the down regulation of atherosclerosis-related genes, the increase of oxLDL autoantibodies or the resistance of isolated LDL, after administration of olive oil, to mediated *in vitro* oxidation. The fact that Tyr and its conjugated metabolites bind to LDL, as is the case of Htyr and metabolites, renders possible a protective effect according to literature. Despite the lack of *in vivo* data, experiments based on cell-mediated oxidation of LDL showed that Tyr provided a 40% inhibition and preserved the antioxidant defense probably due to its intracellular accumulation, whereas it could protect Caucasian colon adenocarcinoma (Caco)-2 cells from injury induced by oxLDL.

4 Proposal for a consensus

It is clear from all the above evidence that the health claim on “olive oil polyphenol” refers to both tyrosol and hydroxytyosol, free or in bound forms. A consensus among all the interested parties will facilitate the development and the adoption of appropriate analytical methods for the determination of all the phenolic compounds that should be quantified.

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Conflict of interest

The authors state that there is no conflict of interest

References

[1] EC Regulation No. 432/2012 of 16 May 2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk, to children's development, health. *Off. J. Eur. Comm.* **2012**, *L136*, 1.

[2] EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA. Scientific Opinion on the substantiation of health claims related to polyphenols in olive and protection of LDL particles from oxidative damage (ID 1333, 1638, 1639, 1696, 2865), maintenance of normal blood HDL-cholesterol concentrations (ID 1639), maintenance of normal blood pressure (ID 3781), "anti-inflammatory properties" (ID 1882), "contributes to the upper respiratory tract health" (ID 3468), "can help to maintain a normal function of gastrointestinal tract" (3779), and "contributes to body defenses against external agents" (ID 3467) pursuant to Article 13(1) of Regulation (EC) No 1924/2006, *EFSA J.* **2011**, *9*, 2033.

[3] M. Z. Tsimidou, D. Boskou, The health claim on "olive oil polyphenols" and the need for meaningful terminology and effective analytical protocols. *Eur. J. Lipid Sci. Technol.* **2015**, *117*, 1091.

[4] C. Romero, M. Brenes, Analysis of total contents of hydroxytyrosol and tyrosol in

olive oils. *J. Agric. Food Chem.* **2012**, *60*, 9017.

[5] A. Mastralexi, N. Nenadis, M. Z. Tsimidou, Addressing analytical requirements to support health claims on "olive oil polyphenols" (EC regulation 432/2012). *J. Agric. Food Chem.*, **2014**, *62*, 2459 (C. Romero, M. Brenes, Comment on addressing analytical requirements to support health claims on “olive oil polyphenols” (EC regulation 432/212). *J. Agric. Food Chem.* **2014**, *62*, 10210; A. Mastralexi, N. Nenadis, M. Z. Tsimidou, Rebuttal to the comment on addressing analytical requirements to support health claims on “olive oil polyphenols” (EC regulation 432/212). *J. Agric. Food Chem.* **2014**, *62*, 10212.

[6] G. Purcaro, R. Codony, L. Pizzale, C. Mariani, L. Conte, Evaluation of total hydroxytyrosol and tyrosol in extra virgin olive oils. *Eur. J. Lipid Sci. Technol*. **2014**, *116*, 805.

[7] P. Reboredo-Rodríguez, E. Valli, A. Bendini, G. Di Lecce, J. Simal-Gándara, T. GallinaToschi, A widely used spectrophotometric assay to quantify olive oil biophenols according to the health claim (EU Reg. 432/2012). *Eur. J. Lipid Sci. Technol*. **2016**, *118*, 1593.

[8] M. Ricciutelli, S. Marconi, M. C. Boarelli, G. Caprioli, G. Sagratini, R. Ballini, D. Fiorini, Olive oil polyphenols: A quantitative method by high-performance liquid-chromatography-diode-array detection for their determination and the assessment of the related health claim. *J. Chromatogr.* A, **2017**, *1481*, 53.

[9] L. Bartella, F. Mazzotti, A. Napoli, G. Sindona, L. Di Donna, A comprehensive evaluation of tyrosol and hydroxytyrosol derivatives in extra virgin olive oil by microwave-assisted hydrolysis and HPLC-MS/MS. *Anal. Bioanal. Chem.* **2018**, *410*, 2193.

[10] F. Alagna, R. Mariotti, F. Panara, S. Caporali, S. Urbani, G. Veneziani, S.

Esposto, A. Taticchi, A Rosati, R. Rao, G. Perrotta, M. Servili, L. Baldoni, Olive phenolic compounds: Metabolic and transcriptional profiling during fruit development. *BMC Plant Biol*. **2012**, *12*, art. no. 162 (and ref.1, 8, 49, 59).

[11] P. Inglese, F. Famiani, F. Galvano, M. Servili, S. Esposto, S. Urbani, Factors affecting extra-virgin olive oil composition, in *Horticultural Reviews*, Vol 38 (Ed: Jules Janik), John Wiley & Sons, Inc., Hoboken, NJ, USA, **2011**, Ch. 3.

[12] M. Tsimidou, Polyphenols and quality of virgin olive oil in retrospect. *Ital. J. Food Sci*. **1998**, *10*, 99.

[13] A. Bendini, L. Cerretani, A. Carrasco-Pancorbo, A. M. Gómez-Caravaca, A. Segura-Carretero, A. Fernández-Gutiérrez, G. Lercker, Phenolic molecules in virgin olive oils: A survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade. *Molecules* **2007**, *12*, 1679.

[14] M. Servili, S. Esposto, R. Fabiani, S. Urbani, A. Taticchi, F. Mariucci, R. Selvaggini, G. F. Montedoro, Phenolic compounds in olive oil: antioxidant, health and organoleptic activities according to their chemical structure. *Inflammopharmacol.* **2009**, *17*, 76.

[15] D. Boskou, *Olive and Olive Oil Bioactive Ingredients*, AOCS Press, Urbana, Il, **2015**.

[16] S. Christophoridou, P. Dais, L. I. H. Tseng, M. Spraul, Separation and identification of phenolic compounds in olive oil by coupling high-performance liquid chromatography with post-column solid-phase extraction to nuclear magnetic resonance spectroscopy (LC-SPE-NMR). *J. Agric. Food Chem.* **2005**, *53*, 4667.

[17] M. Pérez-Trujillo, A. M. Gómez-Caravaca, A. Segura-Carretero, A. Fernández-

Gutiérrez, T. Parella, Separation and identification of phenolic compounds of extra virgin olive oil from *Olea europaea* L. by HPLC-DAD-SPE-NMR/MS. Identification of a new diastereoisomer of the aldehydic form of oleuropein aglycone. *J. Agric. Food Chem.* **2010**, *58*, 9129.

[18] P. Diamantakos, A. Velkou, B. K. Killday, T. Gimisis, E. Melliou, P. Magiatis, Oleokoronal and oleomissional: new major phenolic ingredients of extra virgin olive oil. *Olivae*, **2015**, 122.

[19] International Olive Council, Determination of biophenols in olive oils by HPLC, COI/T.20/Doc No 29 November **2009**.

[20] A. Bianco, M. A. Chiacchio, G. Grassi, D. Iannazzo, A. Piperno, R. Romeo, Phenolic components of *Olea europea*: Isolation of new tyrosol and hydroxytyrosol derivatives. *Food Chem.* **2006**, *95*, 562.

[21] A. Bianco, F. Coccioli, M. Guiso, C. Marra, The occurrence in olive oil of a new class of phenolic compounds: hydroxy-isochromans. *Food Chem*. **2001**, *77*, 405.

[22] A. Bianco, R. A. Mazzei, C. Melchioni, G. Romeo, M. L. Scarpati, A. Soriero, N. Uccella, Microcomponents of olive oil-III. Glucosides of 2(3,4-dihydroxy-phenyl)ethanol. *Food Chem*. **1998**, *63*, 461.

[23] C. Romero, M. Brenes, P. García, A. Garrido, Hydroxytyrosol 4-β-D-glucoside, an important phenolic compound in olive fruits and derived products. *J. Agric. Food Chem*. **2002**, *50*, 3835.

[24] M. N. Vissers, P. L. Zock, M. B. Katan, Bioavailability and antioxidant effects of olive oil phenols in humans: a review. *Eur. J. Clin. Nutr.* **2004**, *58*, 955.

[25] M. I. Covas, M. Fitó, R. De la Torre, Minor bioactive olive oil components and health: Key data for their role in providing health benefits in humans, in *Olive and Olive Oil Bioactive Ingredients*, (Ed: D. Boskou), AOCS Press, Urbana, Il, **2015**,

Ch. 2.

[26] M. I., Covas, K. Nyyssönen, H. E. Poulsen, J. Kaikkonen, H. J. F. Zunft, H. Kiesewetter, A. Gaddi, R. De La Torre, J. Mursu, H. Bäumler, S. Nascetti, J. T. Salonen, M. Fitó, J. Virtanen, J. Marrugat, The effect of polyphenols in olive oil on heart disease risk factors: A randomized trial. *Annals Int. Med.* **2006**, *145*, 333.

[27] S. Christophoridou, P. Dais, Detection and quantification of phenolic compounds in olive oil by high resolution ^{1}H nuclear magnetic resonance spectroscopy. *Anal. Chim. Acta* **2009**, *633*, 283.

[28] A. Mastralexi, N. Nenadis, M. Z. Tsimidou, Total hydroxytyrosol and tyrosol of virgin olive oils from different cultivars, **2018** (unpublished data)

[29] D. L. García-González, N. Romero, R. Aparicio, Comparative study of olive oil quality from single varieties cultivated in Chile and Spain. *J. Agric. Food Chem.* **2010**, *58*, 12899.

[30] E. Karkoula, A. Skantzari, E. Melliou, P. Magiatis, 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. *J. Agric. Food Chem.* **2012**, *60*, 11696.

[31] E. Karkoula, A. Skantzari, E. Melliou, P. Magiatis, Quantitative measurement of major secoiridoid derivatives in olive oil using qNMR. Proof of the artificial formation of aldehydic oleuropein and ligstroside aglycon isomers. *J. Agric. Food Chem.* **2014**, *62*, 600.

[32] A. Ben Mansour, B. Gargouri, E. Melliou, P. Magiatis, M. Bouaziz, Oil quality parameters and quantitative measurement of major secoiridoid derivatives in Neb Jmel olive oil from various Tunisian origins using qNMR. *J. Sci. Food Agric.* **2016**, *96*, 4432.

[33] S. Cicerale, L. Lucas, R. Keast, Biological activities of phenolic compounds present in virgin olive oil. *Inter. J. Mol. Sci*. **2010**, *11*, 458.

[34] M. Gómez-Romero, R. García-Villalba, A. Carrasco-Pancorbo, A. Fernández-Gutiérrez, Metabolism and bioavailability of olive oil polyphenols, in *Olive oil - constituents, quality, health properties and bioconversions*, (Ed: D. Boskou), InTech, Rijeka, Croatia, **2012**, Ch.18.

[35] J. Rodríguez-Morató, A. Boronat, A. Kotronoulas, M. Pujadas, A. Pastor, E. Olesti, C. Pérez-Mañá, O. Khymenets, M. Fitó, M. Farré, R. de la Torre, Metabolic disposition and biological significance of simple phenols of dietary origin: hydroxytyrosol and tyrosol. *Drug Metab. Rev*. **2016**, *48*, 218.

[36] J. Rodríguez-Morató, P. Robledo, J. A. Tanner, A. Boronat, C. Pérez-Mañá, C. Y. Oliver Chen, R. F. Tyndale, R. de la Torre, CYP2D6 and CYP2A6 biotransform dietary tyrosol into hydroxytyrosol. *Food Chem.* **2017**, *217*, 716.

[37] O. Castañer, M. Fitó, M. C. López-Sabater, H. E. Poulsen, K. Nyyssönen, H. Schröder, J. T. Salonen, K. De la Torre-Carbot, H. F. Zunft, R. De la Torre, H. Bäumler, A. V. Gaddi, G. T. Saez, M. Tomás, M. I. Covas, The effect of olive oil polyphenols on antibodies against oxidized LDL. A randomized clinical trial. *Clin. Nutr.***2011**, *30*, 490.

[38] A. Hernáez, A. T. Remaley, M. Farràs, S. Fernández-Castillejo, I. Subirana, H. Schröder, M. Fernández-Mampel, D. Muñoz-Aguayo, M. Sampson, R. Solà, M. Farré, R. de la Torre, M. C. López-Sabater, K. Nyyssönen, H. J. F. Zunft, M. I. Covas, M. Fitó, Olive oil polyphenols decrease LDL concentrations and LDL atherogenicity in men in a randomized controlled trial. *J. Nutr.***2015**, *145*, 1692.

[39] S. A. Wiseman, L. B. M. Tijburg, F. H. M. M. Van de Put, Olive oil phenolics protect LDL and spare vitamin E in the hamster, *Lipids*, **2002**, *37*, 1053.

[40] R. Masella, C. Giovannini, R. Varì, R. Di Benedetto, E. Coni, R. Volpe, N. Fraone, A. Bucci, Effects of dietary virgin olive oil phenols on low density lipoprotein oxidation in hyperlipidemic patients. *Lipids*, **2001**, *36*, 1195.

[41] C. Giovannini, E. Straface, D. Modesti, E. Coni, A. Cantafora, M. De Vincenzi, W. Malorni, R. Masella, Tyrosol, the major olive oil biophenol, protects against oxidized-LDL- induced injury in Caco-2 cells. *J. Nutr.* **1999**, *129*, 1269.

[42] R. Di Benedetto, R. Varì, B. Scazzocchio, C. Filesi, C. Santangelo, C. Giovannini, P. Matarrese, M. D'Archivio, R. Masella, Tyrosol, the major extra virgin olive oil compound, restored intracellular antioxidant defences in spite of its weak antioxidative effectiveness. *Nutr. Metab. Cardiovasc. Dis.* **2007**, *17*, 535.

Table 1. Hydroxytyrosol derivatives in olive oil

No	Compound	Peak no according to COI*
1	Hydroxytyrosol/[(3,4-dihydroxyphenyl)ethanol]/ 3,4-DHPEA	1
2	Hydroxytyrosol acetate/4-(Acetoxyethyl)-1,2-dihydroxybenzene	8
3	Oleuropeinaglycone (hydroxylic)	23
4	Aldehydic form of oleuropeinaglycone (2 stereoisomers)	23
5	Dialdehydic form of oleuropeinaglycone/ oleuropeindial	14
6	Enolic tautomer of the dialdehydic form of oleuropeinaglycone	
7	Decarboxymethyl form of oleuropeinaglycone	
8	Dialdehydic form of decarboxymethylelenolic acid linked to 3,4-DHPEA/oleacein	12
9	10-Hydroxy-oleuropein aglycone	
10	10-Hydroxy-decarboxymethyl oleuropeinaglycone	

*peak no according to the elution order in the COI/T.20/Doc 29/2009 for olive oil phenols analysis[19]

Table 2. Tyrosol and derivatives in olive oil

No	Compound	Peak no according to COI*
1	Tyrosol/ [(p-hydroxyphenyl)ethanol])/ *p*-HPEA	2
2	Tyrosol acetate	15
3	Ligstrosideaglycone (hydroxylic)	27
4	Aldehydic form of ligstrosideaglycone/ ligstral (2 stereoisomers)	27
5	Dialdehydic form of ligstrosideaglycone/ligstrodial	20
6	Enolic tautomer of the dialdehydic form of ligstrosideaglycone	
7	Decarboxymethyl form of ligstrosideaglycone	
8	Dialdehydic form of decarboxymethylelenolic acid linked to *p*–HPEA/oleocanthal	17

*peak no according to the elution order in the COI/T.20/Doc 29/2009 for olive oil phenols analysis[19]

Table 3. Molar ratios of Htyr and derivatives to Tyr and derivatives in virgin olive oils from different cultivars analyzed using different methods

Molar ratios								
Htyr/Tyr						**oleacein/ oleocanthal**	**Oleacein + oleuropein aldehyde aglycone /oleocanthal + ligstroside aldehyde aglycone**	
1 *n*=4	**2** *n*=7	**3** *n*=10	**4** *n*=8	**5** *n*=10	**6** *n*=7	**7** *n*=10	**8** *n*=15	**9** *n*=4
1.36a	0.45a	3.10a	1.72	0.21a	1.32	0.39	0.81a	0.80
1.46a	0.36b	1.43a	1.05	0.58b	0.82	1.41	1.14a	0.42
1.054b	1.04c	1.69a	0.90	0.60b	0.79	1.16	0.74b	0.68
1.32b	1.19d	0.60b	0.62	0.82b	1.39	0.96	0.63c	0.89
	0.81e	0.94b	1.85	0.55b	0.79	0.43	0.23c	
	1.01f	2.01c	0.90	1.22b	1.02	0.66	0.38e	
	1.14g	1.50c	1.29	0.77b	0.32	0.94	0.79d	
		0.61c	1.41	0.82b		0.39	0.28b	
		0.33d		0.21c		0.85	0.69a	
		0.44d		0.58d		0.71	0.69a	
							0.67a	
							0.70a	
							0.84a	
							0.59a	
							0.97a	

1 Greek virgin olive oils from Peloponnese (Koroneiki[a], Tsounati[b]) determined by NMR[27]

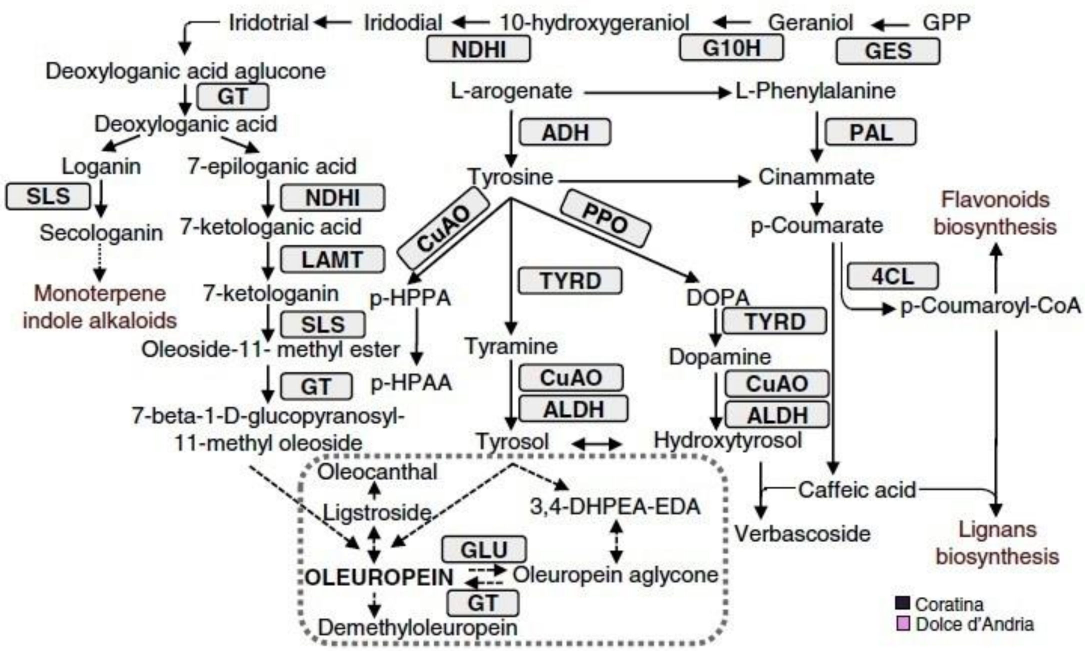
2 Commercial olive oils (mild[a], intensely flavored[b] olive oils , extra virgin olive oil of unknown cultivar[c], Arbequina[d], Manzanilla[e], Hojiblanca[f] and Picual[g]) determined by HPLC-UV after direct application of 2 M HCl acid solution to the oil[4]

3 Tunisian (Zerrari Douirat[a], Chemlali Tataouine[b], Fakhari Douirat[c]) and Greek (Chalkidiki[d]) origin determined by HPLC-UV analysis of the polar fraction prior (determination of free forms) and after acid hydrolysis with 1 M H_2SO_4 (determination of bound forms)[5]

4	Commercial PDO oils determined by GC-FID analysis of the polar fraction after hydrolysis with acetyl chloride and derivatization with N,O-Bis(trimethylsilyl)trifluoroacetamide[6]
5	(Chalkidiki[a], Koroneiki[b], unknown[c], Coratina[d]) determined by HPLC-UV analysis of the polar fraction prior (determination of free forms) and after acid hydrolysis with 1 M H_2SO_4 (determination of bound forms) [28]
6	Samples from Arbequina, Frantoio, Picual, Koroneiki, Barnea, Leccino, Manzanilla cultivars from trees planted in Chile[29]
7	Greek Samples (all of the Koroneiki cultivar) determined by NMR using the polar fraction[30]
8	Greek virgin olive oils (Koroneiki[a], Wild[b], Throuba[c], Thiaki[d], local of Zakynthos[e]) from different regions determined by NMR using the polar fraction[31]
9	Tunisian virgin olive oils (Neb Jemel) of different geographical origin determined by NMR[32]

Nutrient, substance, food or food category	Claim	Conditions of use of the claim	Conditions and/or restrictions of use of the food and/or additional statement or warning	EFSA Journal number	Relevant entry number in the Consolidated List submitted to EFSA for its assessment
Olive oilpolyphenols	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.		2011;9(4):2033	1333, 1638, 1639, 1696, 2865

Figure 1



Iridotrial
Iridodial
10-hydroxygeraniol
Geraniol
GPP
NDHI
G10H
GES
Deoxyloganic acid aglucone
GT
Deoxyloganic acid
L-arogenate
L-Phenylalanine
ADH
PAL
Loganin
7-epiloganic acid
SLS
NDHI
Tyrosine
Cinammate
Secologanin
7-ketologanic acid
CuAO
PPO
p-Coumarate
Flavonoids biosynthesis
LAMT
Monoterpene indole alkaloids
7-ketologanin
p-HPPA
TYRD
DOPA
4CL
SLS
TYRD
p-Coumaroyl-CoA
Oleoside-11- methyl ester
Tyramine
Dopamine
GT
p-HPAA
CuAO
ALDH
CuAO
ALDH
7-beta-1-D-glucopyranosyl-11-methyl oleoside
Tyrosol
Hydroxytyrosol
Oleocanthal
Caffeic acid
Ligstroside
3,4-DHPEA-EDA
Verbascoside
Lignans biosynthesis
GLU
OLEUROPEIN
Oleuropein aglycone
GT
Demethyloleuropein
Coratina
Dolce d'Andria

Figure 2

Figure legends

Figure 1. The health claim on the phenolic compounds of the EC Reg. 432/2012[1]

Figure 2. Metabolic relationships among Htyr, Tyr and other olive phenols (pathways abstracted from Figure 5 of [10]).