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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

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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.

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 per 20 g oil and the benefits of using such a claim for commercial reasons are still not explored enough by stakeholders. Nevertheless, almost at the same period different analytical approaches appear in literature to address this issue.^[4–9]

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)

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, that is, “(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”

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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 number	Journal	Relevant entry number in the consolidated list submitted to EFSA for its assessment
Olive polyphenols	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. The health claim on the phenolic compounds of the EC Reg. 432/2012.^[1]

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 hydrolyzed during processing, as well as 10-hydroxy-oleuropein,^[15] hydroxyisochromans, 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 intended to include only the free form of this monophenol in the calculation of the minimum amount of 5 mg phenols per 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/20 g 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 deacetyxyglycone are absorbed

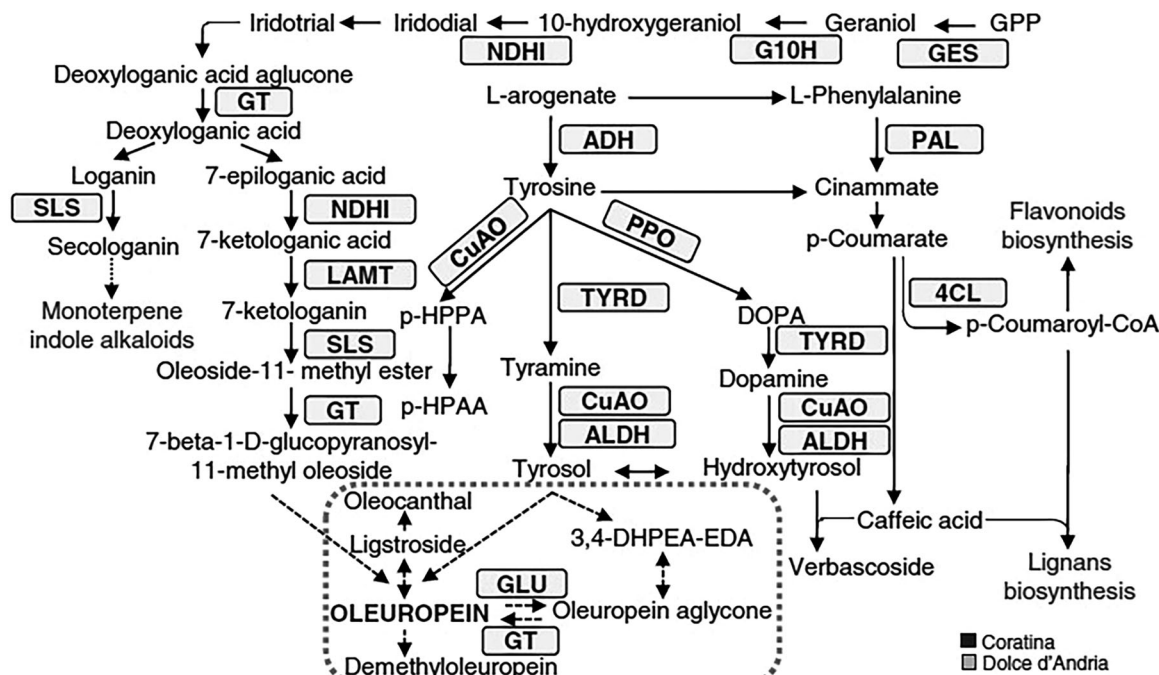


Figure 2. Metabolic relationships among Htyr, Tyr, and other olive phenols (pathways abstracted from Figure 5 of ref.[10]).

and metabolized since their hydrogenated and/or glucuronated derivatives have been detected in human urine after 2 h 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,

Table 1. Hydroxytyrosol derivatives in olive oil.

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

^{a)} Peak no according to the elution order in the COI/T.20/Doc 29/2009 for olive oil phenols analysis.^[19]

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

Table 2. Tyrosol and derivatives in olive oil.

No.	Compound	Peak no according to COI ^{a)}
1	Tyrosol/[(p-hydroxyphenyl)ethanol]/p-HPEA	2
2	Tyrosol acetate	15
3	Ligstroside aglycone (hydroxylic)	27
4	Aldehydic form of ligstroside aglycone/ligstral (2 stereoisomers)	27
5	Dialdehydic form of ligstroside aglycone/ligstrodiol	20
6	Enolic tautomer of the dialdehydic form of ligstroside aglycone	
7	Decarboxymethyl form of ligstroside aglycone	
8	Dialdehydic form of decarboxymethyl elenolic acid linked to p-hPEA/oleocanthal	17

^{a)} 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		
	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 H₂SO₄ (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 H₂SO₄ (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]

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 hydroxytyrosol, 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 declare no conflict of interest.

Keywords

health claim, hydroxytyrosol, olive oil polyphenols, tyrosol

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