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

Maria Z. Tsimidou,* Nikolaos Nenadis, Maurizio Servili, Diego Luis García-González, 
and Tullia Gallina Toschi

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.

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 terminol- 
yogy introduced in the latter. The wording “hydroxytyrosol and derivatives” 
accompanied by an explanation in paren-
thesis “(e.g., oleuropein complex and 
tyrosol)” being not further detailed in the 
EFSA publication triggered several discus-
sions among interested parties regarding 
its unequivocal interpretation.[3] As a 
result, there is a need of clarifica-
tion about which compounds should be summed up 
to give the amount of at least 5 mg phenols 
per 20 g oil and the bene-

fits of using such a 
claim for commercial reasons are still not 
explored enough by stakeholders. Never-
theless, 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 transcrip-
tional 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 


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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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 in virgin olive oil so far using 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.[20] 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.7 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,[14–16,27–32] 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 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, 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

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### Table 1. Hydroxytyrosol derivatives in olive oil.

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Peak no according to COI&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hydroxytyrosol/(3,4-dihydroxyphenyl)ethanol)/3,4-DHPEA</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Hydroxytyrosol acetate/4-(Acetoxethyl)-1,2-dihydroxybenzene</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>Oleuropein aglycone (hydroxycarboxylic)</td>
<td>23</td>
</tr>
<tr>
<td>4</td>
<td>Aldehydic form of oleuropein aglycone (2 stereoisomers)</td>
<td>23</td>
</tr>
<tr>
<td>5</td>
<td>Dialdehydic form of oleuropein aglycone/oleuropeinial</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td>Enolic tautomer of the dialdehydic form of oleuropein aglycone</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Decarboxymethyl form of oleuropein aglycone</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Dialdehydic form of decarboxyethyl elenolic acid linked to 3,4-DHPEA/o!</td>
<td>12</td>
</tr>
<tr>
<td>9</td>
<td>10-Hydroxy-oleuropein aglycone</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10-Hydroxy-decarboxymethyl oleuropein aglycone</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Peak no according to the elution order in the COI/T.20/Doc 29/2009 for olive oil phenols analysis.<sup>[19]</sup>

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### Table 2. Tyrosol and derivatives in olive oil.

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Peak no according to COI&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tyrosol/(p-hydroxyphenyl)ethanol)/p-HPEA</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Tyrosol acetate</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>Ligstroside aglycone (hydroxycarboxylic)</td>
<td>27</td>
</tr>
<tr>
<td>4</td>
<td>Aldehydic form of ligstroside aglycone/ligstral (2 stereoisomers)</td>
<td>27</td>
</tr>
<tr>
<td>5</td>
<td>Dialdehydic form of ligstroside aglycone/ligstrodial</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>Enolic tautomer of the dialdehydic form of ligstroside aglycone</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Decarboxyethyl form of ligstroside aglycone</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Dialdehydic form of decarboxyethyl elenolic acid linked to p-HPEA/o!</td>
<td>17</td>
</tr>
</tbody>
</table>

<sup>a</sup> Peak no according to the elution order in the COI/T.20/Doc 29/2009 for olive oil phenols analysis.<sup>[19]</sup>
Table 3. Molar ratios of Htyr and derivatives to Tyr and derivatives in virgin olive oils from different cultivars analyzed using different methods.

<table>
<thead>
<tr>
<th>Molar ratios</th>
<th>Htyr/Tyr</th>
<th>Oleacein/oleocanthal</th>
<th>Oleacein + oleuropein aldehyde aglycone/oleocanthal + ligstroside aldehyde aglycone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 n = 4</td>
<td>2 n = 7</td>
<td>3 n = 10</td>
</tr>
<tr>
<td>1.36a</td>
<td>0.45a</td>
<td>3.10a</td>
<td>1.72</td>
</tr>
<tr>
<td>1.46a</td>
<td>0.36b</td>
<td>1.43a</td>
<td>1.05</td>
</tr>
<tr>
<td>1.054b</td>
<td>1.04c</td>
<td>1.69a</td>
<td>0.90</td>
</tr>
<tr>
<td>1.32b</td>
<td>1.19d</td>
<td>0.60b</td>
<td>0.62</td>
</tr>
<tr>
<td>0.8le</td>
<td>0.94b</td>
<td>1.85</td>
<td>0.55b</td>
</tr>
<tr>
<td>1.0f</td>
<td>2.01c</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>1.14g</td>
<td>1.50c</td>
<td>1.29</td>
<td>0.77b</td>
</tr>
<tr>
<td></td>
<td>0.6lc</td>
<td>1.41</td>
<td>0.82b</td>
</tr>
<tr>
<td></td>
<td>0.44d</td>
<td>0.58d</td>
<td>0.71</td>
</tr>
</tbody>
</table>

1 Greek virgin olive oils from Peloponnese (Koroneiki, Tsounati) determined by NMR.  
2 Commercial olive oils (mild, intensely flavored) olive oils, extra virgin olive oil of unknown cultivar, Arbequina, Manzanilla, Hojiblanca, and Picual determined by HPLC-UV after direct application of 2 M HCl acid solution to the oil.  
3 Tunisian (Zerrari Douirat, Chemlali Tataouine, Bakhari Douirat) and Greek (Chalkidiki) 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).  
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.  
5 Chalkidiki, Koroneiki, unknown, Coratina 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).  
6 Greek Samples (all of the Koroneiki cultivar) determined by NMR using the polar fraction.  
7 Greek virgin olive oils (Koroneiki, Wild, Throuba, Thiaki, local of Zakynthos) from different regions determined by NMR using the polar fraction.  
8 Tunisian virgin olive oils (Neb Jemel) of different geographical origin determined by NMR.
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.

Acknowledgement

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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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[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.
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