Health claims and bioactive virgin olive phenols. Legal requirements and analytical concerns

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a health claim is any statement about a relationship between food and health


REG.(EC) No 1924/2006 on nutrition and health claims made on foods
• The Commission authorises different health claims provided they are based on scientific evidence and can be easily understood by consumers.

• The European Food Safety Authority (EFSA) is responsible for evaluating the scientific evidence supporting health claims.

• There are different types of health claims.

• Any one of them states, suggests or implies that a relationship exists between a food category, a food, or one of its constituents and health (art 2.2 (5))
Article 13 (1a-c). Health Claims other than those referring to the reduction of disease and the children’s development and health (“general function”)

(a) the role of a nutrient or other substance in growth, development and the function of the body

If it is

(i) Based on generally accepted scientific evidence

(ii) Well understood by the average consumer
For the needs of the regulation

Average consumer
reasonably well-informed
and
reasonably observant and circumspect
taking into account social, cultural and linguistic factors
as interpreted by the Court of Justice,
but makes provision
to prevent the exploitation of consumers
whose characteristics make them
particularly vulnerable to misleading claims
Under article 13(3a) of the above regulation a list of health claims was provisioned and realised by the subsequent regulation Reg (EC) no 432/2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children’s development and health.

- 44,000 claims were scrutinized by the Commission
- 4,637 were submitted to EFSA and examined as prioritized by the EC.
- Evaluation was completed by June 2011
- 341 opinions on 2,758 “general function” health claims were issued
- 74 claims for microorganisms and 17 ones for insufficient evidence for further examination (from MS to EFSA through the EC)

All of them can be found in a Register that includes information on the permitted nutrition claims, a list of authorized and rejected health claims and information on health claims for which the authorization procedure is in progress.

**** after consulting EFSA
Criteria for the initial screening of Article 13 (3) health claims of Regulation (EC) No 1924/2006
Agreed by the NDA panel on 7 October 2008

Criteria for returning health claims back to the Commission for further clarification on the scope or information to be added for:

- Claims where clarification on scope is needed (e.g. claims referring to risk reduction or referring to children’s development and health, or medicinal claims)
- General well-being claims where the health relationship is not clear, e.g. “Compound X supplementation to sustain vitality while aging”
- Claims which are too vague (claim effect not specified/measurable), e.g. Compound X and “energy and vitality”. Proposed wording: Compound X is “necessary to maintain energy and general vitality”
- Foods which are not sufficiently characterised or conditions of use are not sufficiently specified
- Combination constituents that are not sufficiently defined
- Claims in other languages than English (to be returned for translation). If EFSA is asked to carry out the translations, EFSA will send translated claims back to Member States for validation of the translation.
Among those re-evaluated

A “general function” health claim related to olive oil polyphenols was included
SCIENTIFIC OPINION

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 defences against external agents” (ID 3467) pursuant to Article 13(1) of Regulation (EC) No 1924/2006

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)

European Food Safety Authority (EFSA), Parma, Italy

SUMMARY

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to provide a scientific opinion on a list of health claims pursuant to Article 13 of Regulation (EC) No 1924/2006. This opinion addresses the scientific substantiation of health claims in relation to polyphenols in olive and protection of LDL particles from oxidative damage, maintenance of normal blood HDL-cholesterol concentrations, maintenance of normal blood pressure, “anti-inflammatory properties”, “contributes to the upper respiratory tract health”, “can help to maintain a normal function of gastrointestinal tract”, and “contributes to body defences against external agents”. The scientific substantiation is based on the information provided by the Member States in the consolidated list of Article 13 health claims and references that EFSA has received from Member States or directly from stakeholders.
SCIENTIFIC OPINION

Scientific Opinion on the substantiation of health claims related to olive oil and maintenance of normal blood LDL-cholesterol concentrations (ID 1316, 1332), maintenance of normal (fasting) blood concentrations of triglycerides (ID 1316, 1332), maintenance of normal blood HDL-cholesterol concentrations (ID 1316, 1332) and maintenance of normal blood glucose concentrations (ID 4244) pursuant to Article 13(1) of Regulation (EC) No 1924/2006

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European Food Safety Authority (EFSA), Parma, Italy

SUMMARY

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to provide a scientific opinion on a list of health claims pursuant to Article 13 of Regulation (EC) No 1924/2006. This opinion addresses the scientific substantiation of health claims in relation to olive oil and maintenance of normal blood LDL-cholesterol concentrations, maintenance of normal (fasting) blood concentrations of triglycerides, maintenance of normal blood HDL-cholesterol concentrations and maintenance of normal blood glucose concentrations. The scientific substantiation is based on the information provided by the Member States in the consolidated list of Article 13 health claims and references that EFSA has received from Member States or directly from stakeholders.

The food that is the subject of the health claims is olive oil. The Panel considers that olive oil is sufficiently characterised in relation to the claimed effects.
SCIENTIFIC OPINION

Scientific Opinion on the substantiation of a health claim related to polyphenols in olive and maintenance of normal blood HDL-cholesterol concentrations (ID 1639, further assessment) pursuant to Article 13(1) of Regulation (EC) No 1924/2006

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 7 September 2012, replaces the earlier version published on 7 August 2012

ABSTRACT

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to provide a scientific opinion on a health claim pursuant to Article 13 of Regulation (EC) No 1924/2006 in the framework of further assessment related to polyphenols in olive and maintenance of normal blood HDL-cholesterol concentrations. The food constituent, polyphenols in olive (olive fruit, olive mill waste waters or olive oil, *Olea europaea* L. extract and leaf) standardised by their content of hydroxytyrosol and its derivatives (e.g. oleuropein complex), that is the subject of the health claim is sufficiently characterised. The claimed effect, maintenance of normal blood HDL-cholesterol concentrations, which is eligible for further assessment, is a beneficial physiological effect. The proposed target population is the general population. No evidence from which conclusions could be drawn for the scientific substantiation of the claim, in addition to the Panel’s earlier opinion, was provided. The Panel considers that no data were submitted which would require a reconsideration of the conclusions expressed in its previous opinion, in which it concluded that the evidence provided was insufficient to establish a cause and effect relationship between the consumption of olive oil polyphenols (standardised by the content of hydroxytyrosol and its derivatives) and maintenance of normal blood HDL cholesterol concentrations.

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The health claim finally approved in Reg (EC) no 432/2012 is spelled as:

“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.

Furthermore, information is given to the consumer that “the beneficial effect is obtained with a daily intake of 20 g of olive oil”.
Only ID 1638, 1639 concern olive oil polyphenols and olive oil

Reg 432/12

<table>
<thead>
<tr>
<th>Nutrient, substance, food or food category</th>
<th>Claim</th>
<th>Conditions of use of the claim</th>
<th>Conditions and/or restrictions of use of the food and/or additional statement or warning</th>
<th>EFSA Journal number</th>
<th>Relevant entry number in the Consolidated List submitted to EFSA for its assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olive oil polyphenols</td>
<td>Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress</td>
<td>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.</td>
<td></td>
<td>2011:9(4):2033</td>
<td>1333, 1638, 1639, 1694, 2865</td>
</tr>
</tbody>
</table>
we shall focus on each of the parts of this health claim with regards to

1. terminology
2. analytical requirements
Is the term “Olive oil” the most appropriate one?

“Olive oil” is a generic term for the type of oil (from olives) and does not correspond to any of the edible categories authorized by the European Union, the USA or elsewhere.

“Virgin olive oil” (VOO) should be used instead.

Which olive oils are expected to contain high quantities of the phenolic compounds under this health claim?

fresh, extra VOOs obtained from healthy olives of the appropriate maturity stage under optimized olive mill conditions

(two phase systems provide oils with a higher phenolic content, cold pressed oils are expected to contain a higher phenolic content)
Table 1. Major phenolic compounds in VOO

<table>
<thead>
<tr>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>tyrosol</td>
</tr>
<tr>
<td>hydroxytyrosol</td>
</tr>
<tr>
<td>oleuropein aglycons</td>
</tr>
<tr>
<td>ligstroside aglycons</td>
</tr>
<tr>
<td>diacetoxy and dialdehydic forms of oleuropein aglycons</td>
</tr>
<tr>
<td>diacetoxy and dialdehydic forms of ligstroside aglycons</td>
</tr>
<tr>
<td>flavonoids</td>
</tr>
<tr>
<td>phenolic acids, lignans</td>
</tr>
</tbody>
</table>

Is the term “polyphenols” the most appropriate one?
Terminology used in EFSA opinions and REG 432/2012 regarding health claim of virgin olive oil phenolic compounds

REFERENCES


EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2011. 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 defences against external agents” (ID 3467) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal, 9(4):2033, 12 pp.


• the term “polyphenols” - probably deriving from wine phenolic compounds terminology decades ago- does not coincide with the basic structure of the secoiridoids and their simple derivatives present in VOO for which the claim was assigned approved (i.e. hydroxytyrosol and its derivative, e.g. oleuropein complex and tyrosol).
<table>
<thead>
<tr>
<th>Αλκοόλες</th>
<th>R = H</th>
<th>R = OH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tyrosol</strong></td>
<td></td>
<td><strong>Hydroxytyrosol</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Secoiridoids</th>
<th>R = OH</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oleuropein aglycone</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ligstroside aglycone</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ειδικά</th>
<th>R1, 2 = H</th>
<th>R1 = OH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dialdehyde of ligstroside aglycone</strong></td>
<td></td>
<td><strong>Dialdehyde of oleuropein aglycone</strong></td>
</tr>
<tr>
<td><strong>Deacetoxy ligstroside aglycone (oleocanthal)</strong></td>
<td></td>
<td><strong>Deacetoxy oleuropein aglycone (oleacein)</strong></td>
</tr>
</tbody>
</table>
The generic term

- “Virgin olive oil bioactive phenolic compounds” or “VOO phenolic compounds” better expresses the scientific content of the health claim.

- As it informs the average consumer which type of olive oil (the virgin ones) contain some compounds of phenolic nature that are bioactive
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.

“Virgin olive oil bioactive phenols” (i.e. hydroxytyrosol/tyrosol and their bound forms) better express the scientific content of the health claim.
How can I measure this content?

1. Characterisation of the food constituent

The food constituent that is the subject of the health claims is polyphenols (e.g. hydroxytyrosol and oleuropein complex) in olive (olive fruit, olive mill waste waters or olive oil, *Olea europaea* L. extract and leaf).

The conditions of use specify 200 mg/day of polyphenols (ID 1638, 1882, 2865), 2-15 mg per day of hydroxytyrosol or oleuropein complex (ID 1638, 1639, 1696), and 250-500 mg of an *Olea europaea* L. extract standardised to 4-23% oleuropein (ID 3407, 3408, 3779, 3781).

Polyphenols comprise a very wide group (several thousands of compounds) of plant secondary metabolites including flavonoids, isoflavonoids, phenolic acids, proanthocyanidins and other tannins, and lignans with different biological activities. The major polyphenols in olive oil are phenolic acids (e.g. hydroxytyrosol and tyrosol), secoiridoids (e.g. oleuropein) and lignans (e.g. pinenesol). Table olives typically contain hydroxytyrosol, tyrosol, caffeoylquinic acid, verbascoside, luteolin and rutin. Hydroxytyrosol, a major polyphenol typically present in olives, is also present in olive mill waste water. In nature, hydroxytyrosol is found in olives in the form of its cinnamic acid ester, oleuropein. These polyphenolic compounds can be measured in foods by established methods.

Total polyphenols are usually expressed as gallic acid equivalents (GAE), but other phenolic compounds such as catechin/epicatechin or caffeic acid have also been used for standardisation. This standardisation refers to the traditional spectrophotometric measurement of total polyphenols using the Folin-Ciocalteau method (Singleton and Rossi, 1965), which is based on reducing capacity. The method is not specific for polyphenols because other reducing compounds such as ascorbic acid, sugars and proteins will also be included in the quantification, thus leading to an overestimation of the actual polyphenol content. The total polyphenol content assessed with this method is not suitable for characterisation of polyphenols in foods.

The Panel considers that polyphenols (e.g. hydroxytyrosol and oleuropein complex) in olive (olive fruit, olive mill waste waters or olive oil, *Olea europaea* L. extract and leaf) can be characterised by their content of hydroxytyrosol and its derivatives (e.g. oleuropein complex).

The Panel considers that the food constituent, polyphenols in olive (olive fruit, olive mill waste waters or olive oil, *Olea europaea* L. extract and leaf) standardised by their content of hydroxytyrosol and its derivatives (e.g. oleuropein complex), which is the subject of the health claims, is sufficiently characterised in relation to the claimed effects.

Virgin olive oil polyphenols are not several thousands, the same applies for phenolics in olives treated and untreated and olive waste mills.

Which are the established methods? Not clear.

The F-C method is not suitable as it gives an overview of all compounds that can react with the reagent involved but why ascorbic acid, sugars and proteins are referred? Such compounds are not found in the oil matrix. Singleton has reported such interferences for wine matrix.

hydroxytyrosol and its derivatives (e.g. oleuropein complex) are the subject of the health claim.
Which analytical method

Of course not a method that determines the total amount of phenolic compounds such as the Folin-Ciocalteu

One of the many

- LC
- GC
- Capillary electrophoresis
- NMR

procedures found in literature


Though not referred in the regulation or in EFSA opinions

If we focus on a protocol used in many olive oil analysis labs (public or private)
What can be the characteristics of a method that can support such a claim?

- A simple one?
- A sophisticated one?

different approaches have appeared in the literature and the issue is still discussed in different fora
Table 2  *market situation for olive oil sector*

1. The EU accounts for around 80 percent of global olive oil production and a similar share to consumption.

2. The EU is a major exporter of olive oil to countries such as the United States, Brazil, Australia and Japan, and has doubled its exports to 324,000 tonnes in the last 10 years.

3. EU olive oil exports tend to be in bottled form.

4. Worldwide only 2 out of 100 kg fat consumed is olive oil (90% in the Mediterranean area which accounts for the 95% of production)

5. Surplus and demand are in good balance within EU and worldwide. Ending stocks remain at normal levels (5-10% of total amounts)

6. A lower cost stage seems to be difficult in short term so that increase in consumption is expected through quality and marketing policy.

7. Production from the South hemisphere will bring changes in marketing in the near future.
Acidic Hydrolysis according to Mullinaci et al. (Protocol 1):

An aliquot (200 μL) from the polar fraction was mixed with 200 μL of a 1M H₂SO₄ solution. The mixture was maintained in a water bath at 80 °C for 2 h. The procedure was carried out in triplicate. Each hydrolysate was then, diluted with 200 μL of acetonitrile-water 50:50, v/v. The three replicates were combined to obtain a representative hydrolysate. The latter was filtered through a 0.45 μm pore size regenerated cellulose membrane before injection into the chromatograph.

Acidic hydrolysis according to Romero and Brenes (protocol 2):

50 mL of a 2 M HCL were added to 2.5 g VOO into a 100 mL glass bottle that was closed with a polypropylene cap. The mixture was vigorously agitated at 250 rpm in a shaker incubator (model MkX, Stoke Poges, U.K.) at 25 °C for 6h in triplicate for each sample. Finally, 2 mL of the aqueous phase was removed by a plastic pipette from each replicate, mixed to form a representative hydrolysate and filtered through a 0.45 μm regenerated cellulose membrane before injection into the chromatograph.
HPLC Zerrari Douirat (2), 280 nm before and **after** acid hydrolysis

HPLC Zerrari Douirat (2), fluorescence, before and **after** acid hydrolysis
Table 2. Effect of Acidic Hydrolysis Protocols to the Total Htyr and Tyr Content in Extra VOOs of Varying TPP Levels

<table>
<thead>
<tr>
<th>Sample</th>
<th>TPP</th>
<th>Htyr + Tyr&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>protocol 1</th>
<th>protocol 2</th>
<th>protocol 1/protocol 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg caffeic acid/ 20 kg oil</td>
<td>mg/20 g oil</td>
<td>protocol 1</td>
<td>protocol 2</td>
<td>protocol 1/protocol 2</td>
</tr>
<tr>
<td>Z&lt;sub&gt;v&lt;/sub&gt;</td>
<td>6.42</td>
<td>2.34</td>
<td>0.88</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>Z&lt;sub&gt;1&lt;/sub&gt;</td>
<td>5.55</td>
<td>321</td>
<td>3.16</td>
<td>0.94</td>
<td>3.4</td>
</tr>
<tr>
<td>Z&lt;sub&gt;2&lt;/sub&gt;</td>
<td>8.39</td>
<td>278</td>
<td>3.10</td>
<td>1.08</td>
<td>2.9</td>
</tr>
<tr>
<td>C&lt;sub&gt;v&lt;/sub&gt;</td>
<td>7.95</td>
<td>420</td>
<td>1.76</td>
<td>0.76</td>
<td>2.3</td>
</tr>
<tr>
<td>C&lt;sub&gt;1&lt;/sub&gt;</td>
<td>7.80</td>
<td>398</td>
<td>2.92</td>
<td>0.70</td>
<td>4.2</td>
</tr>
<tr>
<td>F&lt;sub&gt;v&lt;/sub&gt;</td>
<td>9.07</td>
<td>454</td>
<td>2.28</td>
<td>0.77</td>
<td>3.0</td>
</tr>
<tr>
<td>F&lt;sub&gt;N&lt;/sub&gt;</td>
<td>7.96</td>
<td>398</td>
<td>2.91</td>
<td>0.84</td>
<td>3.5</td>
</tr>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>2.03</td>
<td>102</td>
<td>3.73</td>
<td>0.53</td>
<td>7.0</td>
</tr>
<tr>
<td>Cha&lt;sub&gt;1&lt;/sub&gt;</td>
<td>3.07</td>
<td>384</td>
<td>3.11</td>
<td>0.51</td>
<td>6.1</td>
</tr>
<tr>
<td>Cha&lt;sub&gt;2&lt;/sub&gt;</td>
<td>7.67</td>
<td>2.57</td>
<td>1.08</td>
<td>2.4</td>
<td></td>
</tr>
</tbody>
</table>

Quantification at 280 nm, <sup>b</sup> Hydroxytyrosol and tyrosol were quantified using respective external calibration curves.

The superiority of protocol 1 was evident in all cases (2 - 7 fold higher levels).
• None of the extra VOOS samples contained the minimum required level of total Htyr and Tyr content (5 mg/20 g oil) although some of them (Z2, Fv, FN, Cv) presented a rather high TPP content, unusual for commercial products.

• Using one standard curve for quantification of both phenols, it was evidenced that the use of Tyr resulted in higher levels in comparison to those using Htyr due to differences in $\varepsilon$ values.

• Even so, the required limit was not achieved for any of the VOO samples.

• Fluorescence detection, which is more sensitive than UV absorption, gave comparable estimations of the total content of Htyr and Tyr (~1.2 - 1.4 fold higher) by means of the two calibration curves.

• Using one standard curve it was evidenced that Htyr resulted in higher levels of total phenols in comparison to those using Tyr due to differences in their fluorescence response.

• Accordingly, some of the samples reached the required limit.
Calibration Curves for Htyr and Tyr under UV (A) and Fluorescence (B) Detection
Is simplification of the profile a realistic approach?

- Having an insight in the **RP-HPLC profiles** and the levels of Htyr and Tyr of the polar fraction prior to hydrolysis it was observed that all of the oils were rich in complex forms of Htyr and Tyr.

- Indeed, the respective ranges prior to hydrolysis were extremely low (Htyr: 0.03-0.5; Tyr: nd -0.43 mg/20 g oil) as expected for fresh oils.

- At this point we have to stress, that calculation on a mass basis introduces a systematic error because the bound forms have a much higher molecular mass compared to those of simple phenols.

- Thus, mean MW of the 10 most known bound forms of Htyr and Tyr is~346 amu.

- If a correction factor is introduced in the quantification using both standards [Htyr: 2.2; Tyr: 2.5] then 9 out of 10 VOO samples satisfied the health claim using UV detection (5.2 -8.9 mg/20 g oil) and all of them when fluorescence was used (5.5 -10.6 mg/20 g oil).
Concluding,

• After many years of consultation a health claim is finally approved for VOO bioactive phenols, all of them derivatives of hydroxytyrosol and tyrosol.

• Regarding terminology used by EFSA and EC we think that to help average consumer the term virgin olive oil should replace olive oil and the term polyphenols should be abandoned.

To further ensure consumer protection and avoid unfair competition in the market a simple, reproducible and undisputable protocol should be adopted.

• Determination of total Htyr and Tyr contents in the VOO polar fraction hydrolysate and introduction of a correction factor in quantification is a solution that has to be considered by IOC and EC. Otherwise the limit set by the EC should be reconsidered.
Thanks to my collaborators

Aspa Mastralexi, chemist, MSc
Nikolaos Nenadis, Dr., Lecturer
Thank you for invitation and attention