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<p>(54) Title: A PRODUCT BASED ON OLIVE OIL, ENRICHED AND SUPPLEMENTED WITH ANTIOXIDANTS</p>		
<p>(57) Abstract</p> <p>The product is enriched and supplemented with additives including organic and inorganic antioxidants. The organic antioxidants include plant-derived polyphenols which are preferably different from those naturally present in olive oil, and optionally vitamins, and said inorganic antioxidants include selenium-based derivatives. The production process includes the following stages: dehydration of the polyphenols, dissolution of the polyphenols in glycerol, mixing, preparation of oily solutions of the vitamins, mixing of the abovementioned compounds and of the inorganic antioxidants with an amount of oil such as to support a dispersion of the enriching substances which is 20 times higher than the concentration which the finished product is to have.</p>		

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## A PRODUCT BASED ON OLIVE OIL, ENRICHED AND SUPPLEMENTED WITH ANTIOXIDANTS

## DESCRIPTION

10

The present invention relates to a product based on olive oil, virgin olive oil or extra-virgin olive oil, which is enriched and supplemented with additives including organic and inorganic antioxidants.

15

The organic antioxidants include plant-derived polyphenols which are preferably different from those naturally present in olive oil, and optionally vitamins. Said vitamins include one or more of the following vitamins: A, E and C, it being possible for

20 vitamin C to be in the form of a semi-synthetic product such as ascorbyl 6-palmitate (vitamin C palmitate). Said inorganic antioxidants include selenium-based derivatives such as selenium oxide or sodium selenite.

25

The invention also relates to a process for the production of olive oil or virgin olive oil or extra-virgin olive oil, which are enriched with complexes as above, this process including the following stages:

- drying the polyphenols for 24 hours in a desiccator;
- dissolving the polyphenols, which have been

30 dehydrated beforehand, in glycerol and mixing - especially with a mixer with asymmetrical paddles - for 6-8 hours in an inert atmosphere at a suitable temperature depending on the formulation chosen;- preparing the oily solutions of the vitamins in

35 proportions which are suitable for the final mass to be produced;- preparing selenium dioxide, or alternatively sodium selenite, in proportions which are suitable for the final mass to be produced;

- preparing a suitable amount of oil (filtered beforehand if extra-virgin or virgin oil) such as to support a dispersion of the enriching substances which is 20 times higher than the concentration which the finished product is to have; placing the oil in a temperature-controlled mixer; switching the mixer on at a low spin speed and at the same time transferring the above components into the oil; next, increasing gradually - over about 24 hours - the stirring speed up to the maximum required speed, according to the type of formulation;
- transferring the above stirred product into a main tank, in which the final intended concentration will be achieved; the product in the main tank is stirred throughout the production cycle until finished, under temperature-controlled conditions.

Further advantageous characteristics and embodiments of the product and of the process according to the invention are defined in the attached claims.

Description of the components constituting the novel formulations.

Virgin olive oil.

The description which follows refers to the attached figures, in which:

Fig. 1 is a histogram representing the antioxidant activity of certain phenols of extra-virgin olive oil, compared with that of standard polyphenols and synthetic antioxidants, such as BHT (Perrin 1992); and

Fig. 2 represents the chemical structure of the main procyanidins present in extract of green tea.

It is known that virgin and extra-virgin olive oil is the only plant oil which naturally contains appreciable amounts of polyphenol substances for consumption. According to recent studies aimed at the qualitative and quantitative determination of the polyphenol compounds present in virgin and extra-virgin olive oil, the total polyphenol content may vary significantly as a function of many factors such as the

type of cultivar, the area of cultivation, how long the olives have been ripening, the storage times and methods, the methods of pressing and squeezing, etc. In particular, for oils from Tuscany harvested up to the end of November, the polyphenol content ranges between 150 and 200 mg/kg, while for samples harvested in December this value is about 135 mg/kg, and for oils obtained from specific areas and/or selected cultivars, the value of total polyphenols can be up to 600 mg/kg (G. Montedoro, "I componenti minori degli oli vergini di oliva e la loro importanza qualitativa", Atti Tavola Rotonda Caratteristiche e Qualità olio Extravergine d'oliva ["Minor components of virgin olive oil and their qualitative importance", Round Table Proceedings on Characteristics and Quality of Extra-Virgin Olive Oil] page 41, pub. IVOT 1994).

By also comparing these results with those relating to Italian oils of different origin, it may reasonably be assumed that the total polyphenol content has an average value of 330 mg/kg for the abovementioned oils.

The main polyphenol compounds present in extra-virgin olive oil, virgin olive oil and olive oil are: tyrosol, hydroxytyrosol and derivatives thereof, while phenolic acids such as caffeic acid and derivatives thereof, flavones such as apigenin, luteolin and glycosides thereof and oleuropein and related hydrolysis products can also be found in smaller amounts.

It has been demonstrated in recent years that besides imparting a pleasant taste, these molecules are largely responsible for the stability of extra-virgin olive oil and virgin olive oil, to which they impart great resistance both to autoxidation and to thermal oxidation, thereby also contributing toward increasing the life of the product.

The biological activity of the polyphenols of *Olea europea* has recently been investigated (J.L. Perrin, "Minor compounds and natural antioxidants of

olives and olive oil" Corps Gras [fats], 1991, 39, 25-32) and the results of a study aimed at evaluating the degree of antioxidant action of polyphenol compounds are reported in the histogram of Fig. 1.

5 From this research, it emerges that, for the same concentration, caffeic acid and hydroxytyrosol (obtained from the spontaneous hydrolysis of oleuropein, one of the main polyphenol compounds of stone fruit) constitute the products of highest  
10 antioxidant activity present in virgin olive oil. From the histogram of Fig. 1 it is moreover seen that hydroxytyrasol demonstrates, for the same molar concentration, an activity which is almost three times  
15 as high as that of the known synthetic antioxidant butyl hydroxytoluene (BHT).

Oleuropein is of advantageous biological activity, having recently been shown to have coronarodilatory, hypoglycaemic and anticholesterolaemic actions (Ficarra P., Ficarra L.,  
20 De Pasquae A., Monteforte M.T., Calabrò M.L. "HPLC analysis of oleuropein and some flavonoids in leaf and bud of *Olea europaea* L.", *Il Farmaco*, 1991, 46, 803-815). The antioxidant effect of oleuropein together with hydroxytyrosol has been studied in vitro also with  
25 respect to one of the atherogenetic risk factors such as the oxidation of low density proteins (LDL). The results demonstrate that, at concentrations of  $10^{-5}$  M, the polyphenols show antioxidant activity with respect to LDLs (Visioli F., Galli C., "Oleuropein protects low  
30 density lipoproteins from oxidation", *Life Science*, 1994, 55, 1965-1971).

By means of studies carried out on controlled population samples, it has moreover been demonstrated that the daily consumption of virgin olive oil, typical  
35 of Mediterranean countries, guarantees a supply of natural antioxidants in the diet, which has been correlated with a reduced incidence of cardiovascular risk (E. Bosisio, "Radicali liberi, patologia arteriosclerotica e antiossidanti", *Convegno*

Internazionale Alimenti Mediterranei e Benessere, ["Free radicals, arteriosclerotic pathology and antioxidants", International Congress on Mediterranean Foods and Well-Being], Catania 17-18 November 1995).

5 Many studies have made it possible to make a distinction between different types of edible fat and oncological risk; in particular, experimental studies have demonstrated that, in contrast with corn oil and sunflower oil, virgin olive oil does not increase

10 neoplastic incidence or growth in experimental models with mice. Altogether, the data currently available makes it possible to ascertain that the daily use of virgin olive oil in the diet appears to be associated with a reduction in oncological risk which is greater

15 than the reduction which occurs with polyunsaturated fatty acids (such as oleic, linoleic and linolenic acids) which other vegetable oils are very rich in (A. Giacosa "La riduzione del rischio oncologico", Convegno Internazionale Alimenti Mediterranei e Benessere ["The reduction of oncological risk", International Congress on Mediterranean Foods and Well-Being], Catania 17-18

20 November 1995). On this subject, it should be emphasized that the antioxidant power of virgin olive oil differs markedly from that of other vegetable oils, in particular since virgin olive oil contains higher

25 levels of  $\alpha$ -tocopherol (vitamin E), but above all since it contains appreciable amounts of polyphenolic antioxidants.

#### Polyphenol compounds

30 Many plants such as: *Vitis vinifera*, *Camellia sinensis*, *Camomilla matricaria*, *Camomilla recutita*, plants of the *Citrus* genus, *Cynara scolymus*, *Origanum vulgare*, *Origanum majorana*, *Menta piperita*, *Rosmarinus officinalis*, *Salvia officinalis*, *Rosa canina*, *Thymus*

35 *vulgare*, *Glycine soja* and *Glycine max*, are used for the preparation of natural extracts containing polyphenol compounds with high antioxidant power.

Recently, in the food and dietary field, the extracts most commonly used as sources of polyphenol

compounds have been: extract of procyanidin from the leaves of *Camellia sinensis* (green tea), extract of procyanidin from grapeseeds of *Vitis vinifera*, extract of polyphenols from fruits of the *Citrus* genus, extract of *Cynara scolymus*, etc. Recent studies have demonstrated that among polyphenol compounds, those which exhibit higher antioxidant and anti-free-radical activity are procyanidins and in particular those esterified with gallic acid. Natural extracts containing polyphenol compounds of this nature are those of *Vitis vinifera* and in particular those of *Camellia sinensis* (green tea) which contain, in the largest amount, procyanidins esterified with gallic acid. The main catechins present in the extract of green tea are epigallocatechin 3-gallate, epigallocatechin and epicatechin gallate, the structural formulae of which are given in Fig. 2.

The antioxidant action of this extract was tested recently by the active oxygen method (AOM) by evaluating the level of rancidity of samples of food fats (lard) in comparison with the action of  $\alpha$ -tocopherol (vitamin E) and butyl hydroxyanisole (BHA). Using this test, it was confirmed that the dry extract of procyanidin from green tea is about 20 times as active as vitamin E and about 4 times as powerful as the synthetic antioxidant (BHA) for an identical weight (Yukiniko Hara, "Prophylactic functions of tea polyphenols" from Food Phytochemicals for Cancer Prevention II, Teas, Spices and Herbs Editors Chi-Tang Ho et al., ACS Symposium, Series 547, Am. Chem. Soc. 1994).

Other studies have brought to light the fact that even the individual catechins of green tea are very active and that they act synergistically with ascorbic acid (vitamin C) and with  $\alpha$ -tocopherol (vitamin E) (Matsuzaki T., Hara Y., Nippon Nogeikageku Kaishi, 1985, 59, 129-134). Other studies have demonstrated that the catechins of tea also show antioxidant activity in oils emulsified with water



(Hara Y. "Advances in Food Science and Technology", Nippon Shokuhin Kogyo Gakkay, Korin, Tokyo 1990, 4, 21-39).

### Selenium

5           The understanding of the importance of selenium changed dramatically with the discovery by Schwarz (K. Schwarz, T.M. Foltz, J. Am. Chem. Soc. 1957, 79, 3292) which demonstrates that microamounts of this element act as a protective agent against a type of liver  
10 necrosis induced in laboratory rats fed with diets deficient in selenium, vitamin E and thioamino acids. During the 1980s, particular attention was devoted to selenium as a possible agent for preventing the onset of certain human pathologies and its function as an  
15 essential microelement in the diet has by now been definitively recognized (G.F. Combs and J.S.B. Combs "The role of selenium in nutrition", Academic Press Inc. (London) LTD 1986).

          A daily intake of 50-200 micrograms of selenium  
20 for adults was recommended in 1980 by the Food and Nutrition Board of the US National Academy of Science. The addition of selenium, carried out both on soils with a low content of the element, and on controlled diets, is a widespread practice in many countries in  
25 which a deficiency of this microelement has been demonstrated. For example, in Finland, use has been made of suitable selenium-containing fertilizers to enrich some soils (M. Eurolo, P. Ekholm, M. Ylinen, P. Koivistoinen, P. Varo, Acta Agric. Scand. 1989, 39,  
30 345); moreover, the addition of selenium to the diet has been used in studies aimed at preventing certain types of tumors (C. Ip, H.E. Ganther, Cancer Res., 1990, 50, 1206).

          In recent years, selenium has generated  
35 increasing interest also for its antioxidant and anti-ageing properties and there are many publications on this subject in the literature. Investigations have been carried out to evaluate and compare the antioxidant action of selenium with that of vitamin E

and with that of zinc. In both cases it has been found that the mechanism of the action of selenium as an antioxidant in vivo is different from that of zinc and from that of vitamin E. In particular, it was demonstrated that vitamin E prevents lipid peroxidation more effectively than selenium, while selenium prevents the production of free radicals more effectively than vitamin E (Bettger William, "Zinc and selenium versus general antioxidation", Can. J. Physiol. Pharmacol., 1993, 49, 721-724 - Zhu, L.Z., He, Y.P., Piao, J.H., Cai, Q.Y., Sun, C.P., Chang, J.Z., Wu, K., Cong, J. P. "Difference of antioxidative effects between vitamin E and selenium", Lipid-Soluble Antioxid., 1992, 92-104).

It therefore seems obvious that the simultaneous presence of selenium, vitamin E and polyphenols in a food product can substantially increase its antioxidant activity by way of the synergistic action of the various components present.

Moreover, there are a few food supplements which contain selenium on the Italian market, the selenium concentration in these products ranging between 10 and 80 mg per 100 g of product, combined with variable doses of vitamin E.

#### Vitamins

Information relating to the liposoluble vitamins has not been reported in the present description since their biological activity is widely known, in particular for vitamins A and E which may be incorporated into the formulations according to the invention.

The use of ascorbyl 6-palmitate (vitamin C palmitate) represents an innovative feature since it allows the dissolution in oil of a water-soluble vitamin known for its antioxidant actions (vitamin C). Vitamin C palmitate is moreover present in the list of antioxidants used in the food sector (Tecnoalimenti "Tox Data Food" page 24, pub. OEMF, 1991).

The production process

The process includes the following stages, with reference to the products which are used in the various formulations according to the invention:

- 5 - dehydrating the polyphenols for 24 hours in a desiccator;
- dissolving the dehydrated polyphenol in glycerol; mixing with a mixer with asymmetrical paddles for 6-8 hours under vacuum and at a suitable temperature
- 10 depending on the formulation chosen;
- preparing the oily solutions of the vitamins in proportions which are suitable for the final mass to be produced;
- preparing selenium dioxide, or alternatively sodium
- 15 selenite, in proportions which are suitable for the final mass to be produced;
- preparing a suitable amount of oil (filtered beforehand if extra-virgin or virgin oil) such as to support a dispersion of the enriching substances which
- 20 is 20 times higher than the concentration which the finished product is to have; placing the oil in a temperature-controlled mixer; switching the mixer on at a low spin speed and at the same time transferring the above components into the oil; next, increasing
- 25 gradually - over about 24 hours - the stirring speed up to the maximum required speed, according to the type of formulation;
- transferring the above stirred product into a main tank, in which the final intended concentration will be
- 30 achieved; the product in the main tank is stirred throughout the production cycle until finished, under temperature-controlled conditions.

Example of the preparation of a base formulation with a few possible variants thereof.

- 35 Samples of extra-virgin olive oil were supplemented with the following antioxidants:
- commercial dry extract of procyanidin from green tea
  - commercial dry extract of procyanidin from grapes
  - oleuropein

- caffeic acid
- oily solution of vitamin A
- oily solution of vitamin E
- ascorbyl 6-palmitate (vitamin C palmitate)
- 5 - selenium dioxide or sodium selenite.

Glycerol was used as technological coadjuvant to solubilize the less liposoluble compounds.

The base formulation was prepared in the following way: the extract of procyanidin from the tea  
10 was kept in a desiccator for 24 hours; 40 mg of this extract were dissolved in 0.5-1.0 ml of glycerol and this solution was added to 1 liter of virgin oil, filtered beforehand through a cellulose filter, and maintained under mechanical stirring, at a suitable  
15 temperature. 2 ml of oily solution of vitamin E, equal to 300 mg of d,l- $\alpha$ -tocopheryl acetate, and 1 ml of oily solution of vitamin A, equal to 300,000 I.U., were then added. Lastly, 1.6 mg of selenium dioxide (equivalent to 1.14 mg of selenium) was added and the oil was  
20 maintained under mechanical stirring at a suitable temperature until dissolution was complete.

Various samples containing extracts of procyanidin from green tea were prepared in concentrations between 10 and 40 mg/l. The tests  
25 carried out both on the base formulation and on formulations Nos. 1, 2, 3 and 4 which follow were performed under similar conditions, both with virgin olive oils and with olive oils.

Example of the preparation of Formulation No. 1.

30 Oleuropein powder (predried) was added to the base formulation in concentrations within the range between 5 and 20 mg/l. The oleuropein was added to the glycerol solution of the extract of procyanidin from tea and this mixture was subsequently added to the  
35 sample of extra-virgin olive oil.

Example of the preparation of Formulation No. 2.

Caffeic acid powder (predried) was added to the base formulation in concentrations within the range between 5 and 10 mg/l. The caffeic acid was added to

the glycerol together with the extract of procyanidin from tea and this mixture was subsequently added to the sample of extra-virgin olive oil.

Example of the preparation of Formulation No. 3.

5           A commercial dry extract of procyanidin from grapes, which had been predried, was added to the base formulation in concentrations within the range between 5 and 10 mg/l. This extract was added to the glycerol solution of the extract of procyanidin from tea and  
10 this mixture was subsequently added to the sample of extra-virgin olive oil.

Example of the preparation of Formulation No. 4

15           Ascorbyl 6-palmitate (vitamin C palmitate) was added to the base formulation in concentrations within the range between 10 and 60 mg/l. This product was added to the base formulation of extra-virgin olive oil and the sample was maintained under mechanical stirring at a suitable temperature.

## CLAIMS

1. A product based on olive oil or virgin olive oil or extra-virgin olive oil, which is enriched and supplemented with additives including organic and inorganic antioxidants.
2. The product as claimed in claim 1, in which the organic antioxidants include plant-derived polyphenols which are different from those naturally present in virgin and extra-virgin olive oil, and optionally vitamins.
3. The product as claimed in claim 2, in which said vitamins include one or more of the following vitamins: A, E, C.
4. The product as claimed in claim 3, in which the vitamin C is in the form of a semi-synthetic product such as ascorbyl 6-palmitate (vitamin C palmitate).
5. The product as claimed in claim 1, in which the inorganic antioxidants include selenium-based derivatives.
6. The product as claimed in claim 5, in which said derivatives include selenium oxide or dioxide.
7. The product as claimed in claim 2, in which said plant-derived polyphenols include those of the flavonoid and/or phenolic acid classes, as pure products and/or in combination, and used as more or less purified and/or standardized plant-derived polyphenol complexes.
8. The product as claimed in any one of the preceding claims, in which the additive includes the simultaneous presence of selenium, vitamin E and polyphenols.
9. The product as claimed in claim 2, in which glycerol is used as a technological coadjuvant in order to promote the dissolution of compounds of low liposolubility.
10. Additive complexes as defined in one or more of claims 1 to 9, used as food supplements or dietary products.

11. The complexes as claimed in claim 10, in which the polyphenols are chosen from pure or purified polyphenol extracts obtained from one or more of the following plants: *Vitis vinifera*, *Camellia sinensis*,  
5 plants of the *Citrus* genus, *Camomilla matricaria* and *Camomilla recutita*, *Cynara scolymus*, *Origanum vulgare* and *Origanum majorana*, *Menta piperita*, *Salvia officinalis*, *Rosa canina*, *Thymus vulgaris*, *Glycine soja* and *Glycine max*.
- 10 12. The complexes as claimed in either of claims 10 and 11, in which natural plant-derived polyphenols, selenium derivatives and ascorbyl 6-palmitate (vitamin C palmitate) are added, alone or in combination, to olive oil, virgin olive oil or extra-virgin olive oil.
- 15 13. The complexes as claimed in at least one of claims 10 to 12, in which a base formulation includes commercial dry extract of procyanidin from green tea; commercial dry extract of procyanidin from grapes; oleuropein; caffeic acid; oily solution of vitamin A;  
20 oily solution of vitamin E; ascorbyl 6-palmitate (vitamin C palmitate); selenium dioxide or sodium selenite and glycerol as a coadjuvant to dissolve the poorly liposoluble compounds.
14. A process for the production of olive oil,  
25 virgin olive oil or extra-virgin olive oil which are enriched with complexes according to one or more of the preceding claims, this process including the following stages:
- dehydrating the polyphenols;
  - 30 - dissolving the dehydrated polyphenols in glycerol; mixing for 6-8 hours under vacuum at a suitable temperature depending on the formulation chosen;
  - preparing the oily solutions of the vitamins in proportions which are suitable for the final mass to be  
35 produced;
  - preparing selenium dioxide, or alternatively sodium selenite, in proportions which are suitable for the final mass to be produced;

- preparing a suitable amount of oil (filtered beforehand if extra-virgin or virgin oil) such as to support a dispersion of the enriching substances which is 20 times higher than the concentration which the finished product is to have; placing the oil in a temperature-controlled mixer; switching the mixer on at a low spin speed and at the same time transferring the above components into the oil; next, increasing gradually - over about 24 hours - the stirring speed up to the maximum required speed, according to the type of formulation;

- transferring the above stirred product into a main tank, in which the final intended concentration will be achieved; the product in the main tank is stirred throughout the production cycle until finished, under temperature-controlled conditions.

15. The process as claimed in claim 14, in which, for the preparation of a base formulation: the extract of procyanidin from green tea is kept in a desiccator for 24 hours; about 40 mg of said extract are then dissolved in 0.5-1 ml of glycerol and the solution is added to one liter of virgin oil, which has been prefiltered through cellulose filters and has been kept stirring; 2 ml of oily solution of vitamin E, equal to 300 mg of d,l- $\alpha$ -tocopheryl acetate, and 1 ml of oily solution of vitamin A, equal to 300,000 I.U., are then added; 1.6 mg of selenium dioxide (equivalent to 1.14 mg of selenium) are then added and the oil is kept stirring until dissolution is complete.

16. The process as claimed in claim 15, in which oleuropein powder (predried) is added to the base formulation in concentrations between 5 and 20 mg/l, the oleuropein being added to the glycerol solution of extract of procyanidin from the tea and this mixture being added to the sample of oil.

17. The process as claimed in claim 15, in which caffeic acid powder (predried) is added to the base formulation in concentrations between 5 and 10 mg/l, the caffeic acid being added to the glycerol solution



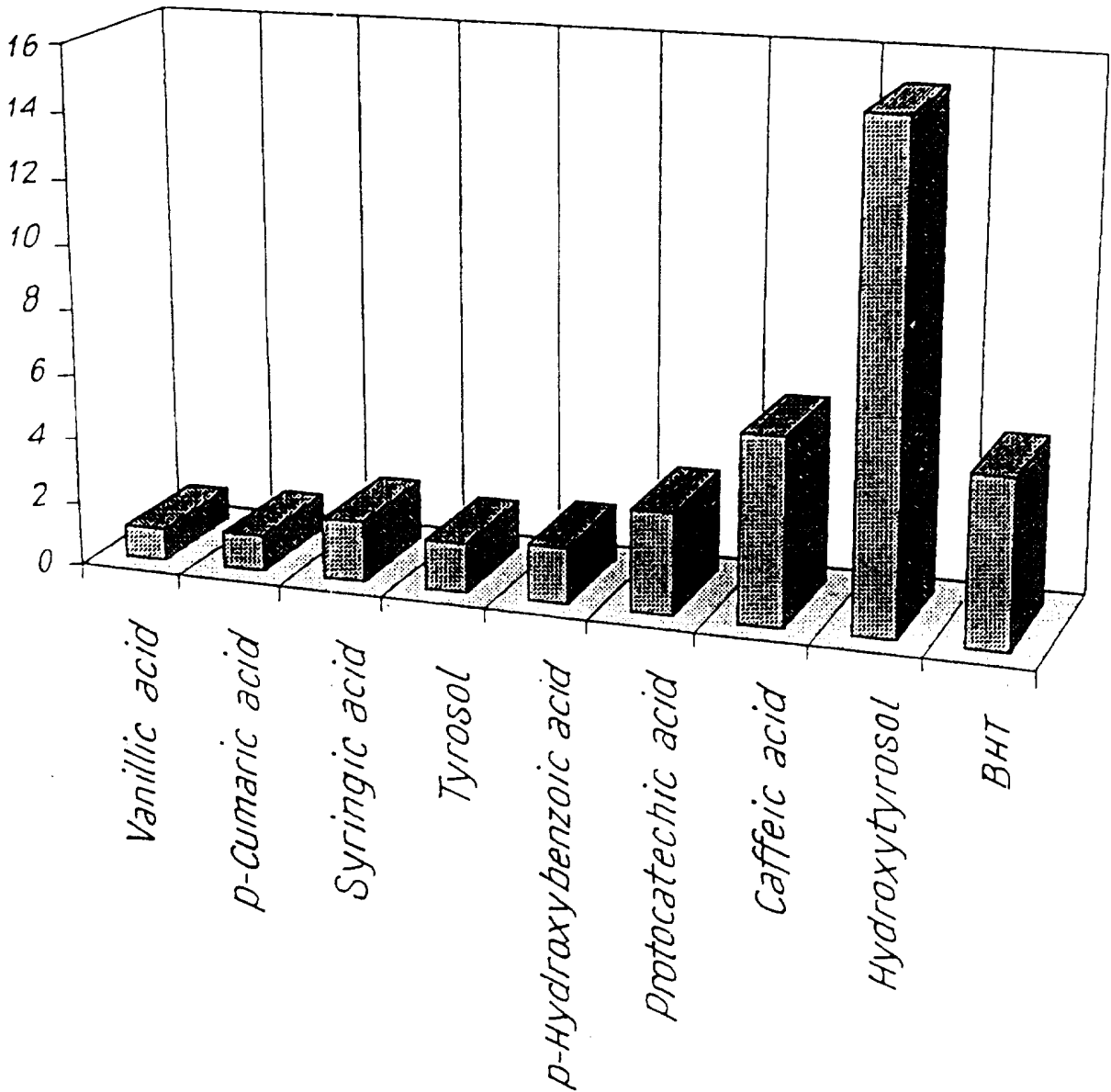
of the extract of procyanidin from the tea and this mixture being added to the sample of oil.

18. The process as claimed in claim 15, in which a commercial dry extract of procyanidin from grapes  
5 (which is predried) is added to the base formulation in concentrations between 5 and 10 mg/l, this extract being added to the glycerol solution of procyanidin from the tea and this combination being added to the sample of oil.

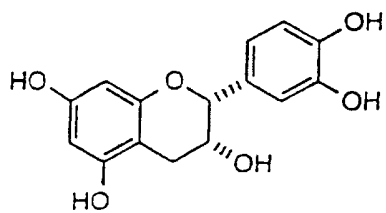
10 19. The process as claimed in claim 15, in which ascorbyl 6-palmitate (vitamin C palmitate) is added to the base formulation in concentrations between 20 and 60 mg/l, the sample being added to the sample of oil, which is kept stirring at a suitable temperature.

15 20. A product based on olive oil or virgin olive oil or extra-virgin olive oil, which is enriched and supplemented with additives including organic and inorganic antioxidants, and a method for the preparation of this product; the whole being as  
20 described above.

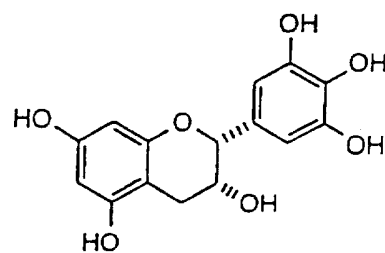
Fig. 1



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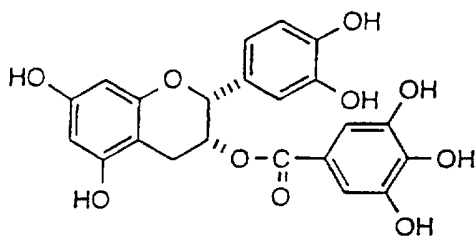


*I (-) Epicatechin*

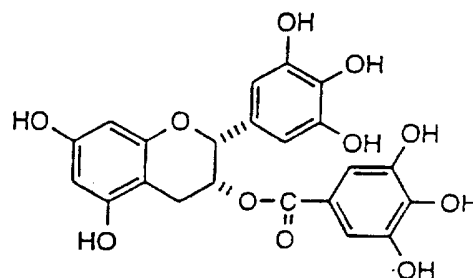


*II (-) Epigallocatechin*

**Fig.2**



*III (-) Epicatechin 3-gallate*



*IV (-) Epigallocatechin 3-gallate*

# INTERNATIONAL SEARCH REPORT

Inter. Application No  
PCT/IT 97/00046

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 C11B5/00 A23D9/06 A23L1/304 A23L1/302 A23L3/3472  
A23L3/3544

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 C11B A23D A23L C09K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 507 064 A (NESTLE SA) 7 October 1992  see page 2, column 2, line 21 - line 36 see page 2, column 2, line 47 - line 53 see claims 1,4,5	1,2,7, 10-12,20
X	---	
X	US 5 444 054 A (GARLEB KEITH A ET AL) 22 August 1995  see column 18, line 56 - line 59 see column 21, line 1 - line 14 see claims 1,8,10,14,15	1,5
Y	---	
	-/--	
		2,3,7, 11,12,14

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

\* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*&\* document member of the same patent family

Date of the actual completion of the international search

15 July 1997

Date of mailing of the international search report

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Dekeirel, M

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/IT 97/00046

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>WO 94 22322 A (KALAMAZOO HOLDINGS INC) 13 October 1994 see page 1, line 22 - line 28 see page 4, line 7 - page 7, line 26 see page 8, line 26 - page 9, line 2 see page 9, line 30 - line 33 see examples 2,3,6</p> <p style="text-align: center;">---</p>	2,3,7, 11,12,14
X	<p>JOURNAL OF THE AMERICAN OIL CHEMISTS' SOCIETY, vol. 70, no. 5, 1 May 1993, pages 483-487, XP000372585 VEKIARI S A: "OREGANO FLAVONOIDS AS LIPID ANTIOXIDANTS" see page 483, Summary see page 484, column 2, last paragraph - page 487, column 2, paragraph 1</p> <p style="text-align: center;">---</p>	1,2,7, 10-12,20
X	<p>DATABASE WPI Section Ch, Week 8725 Derwent Publications Ltd., London, GB; Class A96, AN 87-174722 XP002035223 &amp; JP 62 106 837 A (TAKEDA CHEM IND LTD) , 18 May 1987 see abstract</p> <p style="text-align: center;">---</p>	1,10,20
X	<p>US 5 352 695 A (N GUYEN LAN QUANG ET AL) 4 October 1994 see column 2, line 7 - column 3, line 42</p> <p style="text-align: center;">---</p>	1,10,20
X	<p>REVUE FRANCAISE DES CORPS GRAS, vol. 35, no. 8/9, 1988, PARIS FR, pages 339-344, XP002035222 H. CHIMI ET AL.: "Contribution à l'étude comparative des pouvoirs antioxydants dans l'huile d'olive du tyrosol, de l'hydroxytyrosol, de l'acide caféique, de l'oleuropéine et du B.H.T." see the whole document</p> <p style="text-align: center;">---</p>	1,10,20
A	<p>WO 94 22321 A (KALAMAZOO HOLDINGS INC) 13 October 1994 see examples 1,2,6 see claims 1,7,8,10,12</p> <p style="text-align: center;">---</p>	1-3,7, 10-12,20
A	<p>US 5 059 437 A (TODD JR PAUL H) 22 October 1991 see column 3, line 19 - column 4, line 68 see claims 3-5</p> <p style="text-align: center;">---</p>	1-4,7, 10-12,20
A	<p>GB 2 280 449 A (CHARLEVILLE RES) 1 February 1995 see claims 1,21-23,33,49,50</p> <p style="text-align: center;">---</p>	1-4,10, 12,20
	-/--	

1  
3

# INTERNATIONAL SEARCH REPORT

Inter. Application No.  
PCT/IT 97/00046

**C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4 673 530 A (HARA YUKIHIKO) 16 June 1987 see column 2, line 44 - line 61 see column 6, line 3 - line 8 see figure 3 <p style="text-align: center;">-----</p>	2,7

1

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IT 97/00046

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0507064 A	07-10-92	AT 123209 T	15-06-95
		AU 650951 B	07-07-94
		AU 1124192 A	01-10-92
		CA 2061980 A	01-10-92
		DE 69202716 D	06-07-95
		DE 69202716 T	12-10-95
		ES 2073794 T	16-08-95
		HU 64451 A	28-01-94
		JP 5153932 A	22-06-93
		TR 25887 A	01-11-93
		US 5525260 A	11-06-96
-----			
US 5444054 A	22-08-95	AU 1884495 A	23-10-95
		CA 2187628 A	12-10-95
		EP 0754001 A	22-01-97
		NZ 281878 A	24-02-97
		WO 9526646 A	12-10-95
-----			
WO 9422322 A	13-10-94	NONE	
-----			
US 5352695 A	04-10-94	FR 2675692 A	30-10-92
		AT 119028 T	15-03-95
		AU 1507692 A	29-10-92
		CA 2066924 A	25-10-92
		DE 69201510 D	06-04-95
		DE 69201510 T	13-07-95
		EP 0511118 A	28-10-92
		ES 2069970 T	16-05-95
		JP 5271048 A	19-10-93
		-----	
WO 9422321 A	13-10-94	CA 2159465 A	13-10-94
		EP 0692934 A	24-01-96
		US 5527552 A	18-06-96
-----			
US 5059437 A	22-10-91	AT 124847 T	15-07-95
		CA 2042542 A	17-11-91
		DE 69111234 D	17-08-95
		DE 69111234 T	16-11-95
		EP 0528972 A	03-03-93
		JP 5508317 T	25-11-93

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IT 97/00046

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5059437 A		WO 9117673 A	28-11-91
GB 2280449 A	01-02-95	WO 9619114 A	27-06-96
		IE 940534 A	11-01-95
		IE 940535 A	11-01-95
		AU 1326995 A	10-07-96
US 4673530 A	16-06-87	JP 1044234 B	26-09-89
		JP 1561043 C	31-05-90
		JP 59219384 A	10-12-84