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## Highly Pure Biophenols from Olive Mill Waste (Ageraopherols™)

*A New Natural Molecular Imprinting (NMI™) Extract*

	<p><i>The olive tree (Olea europaea L.) belongs to the botanical family of Oleaceae that includes two botanical varieties: Olea europaea sativa and Olea europaea Oleaster. The olive tree is an evergreen tree; the foliage takes on different aspects depending on the type of farming. The fruit is a drupe whose size and shape depend on the cultivar and growing conditions, and whose weight, physiological maturity, ranging from 1.5 to 4.5 grams.<sup>1</sup></i></p>
<b>Category</b>	Antioxidant, preservatives, functional ingredient
<b>Active constituents</b>	Bio-phenols
<b>Mechanism of activity</b>	Reduction of oxydative stress
<b>Functional Properties<sup>1</sup></b>	<ul style="list-style-type: none"><li>• Antioxidant</li><li>• Inhibition LDL oxydation</li><li>• Antiinflammatory</li><li>• Antibacterial</li><li>• Chemopreventive: Colon and Prostate cancer prevention</li><li>• Coronary heart disease and stroke prevention</li><li>• Atherosclerosis prevention</li></ul>
<b>Applications</b>	Food, Food supplements, Cosmetics and Herbal Drugs
<b>Appearance</b>	Powder green-brownish
<b>Side effects and toxicity</b>	None at the suggested dosage of 250 mg/day



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## GENERAL INFORMATIONS

Olive oil from *Olea europea* drupes consists of triglycerides of oleic, palmitic and linoleic acid, stearin and fatty acids, is used as food for gallbladder kinetic stimulation and laxative properties and is used as an emollient<sup>1</sup>.

It has a high nutrient power, but it is low in vitamin A and is devoid of vitamins B and C. It is easily and completely digestible and its use in food is also advisable, compared with the animal fats, for the high content of unsaturated fatty acids, which have little atherosclerotic action. The best olive oil is obtained by single cold pressure of drupes. Other methods using centrifugation or other mechanical procedure are also permitted.

The pressing of olives produces, in addition to oil, two products of different value: the olive residues and waste water mill. The mill waste water is composed by water initially present in the drupes (vegetation water), by water added to the process to facilitate the separation of oil during the milling and water washing of olives and installations. The mill waste water has a high organic and saline load (8-20% dry weight). The main components are organic sugar, pectin, mucilage, tannins, organic acids, polyols, lipids, nitrogen compounds, polyphenols and a dark pigments ("catecholmelaninic" polymers).

Catecholmelanine and polyphenols are not originally present in this form in olive, but are produced during the mill process in presence of water by means of drupe enzymes, present but inactive in the whole fruit. Just the high presence of a wide variety of bioactive of phenolic components in olive, and products derived there from, could be the key to the economic valorization of waste from oil industry. The glycosylated polyphenols (biophenols) here illustrated derive from the treatment of waste water in the food industry, in particular those resulting, but not limited to, the production of olive oil (vegetation water or waste water mill). The process consists in the selective extraction of glycosylated components. This extract is composed by a particular selected mixture of biophenols (termed *ageraopherols*<sup>TM</sup> by the antiaging effects they express) with very high antioxidant capacity (Orac > 20.000  $\mu\text{mol TE eq/gr}$ ).

## NMI<sup>TM</sup> PHYTO-COSTITUENTS

The main components identified in the extract are glycosylated: Tyrosol, Hydroxytyrosol, Rutin, Luteolin, Neoluteolin, Apigenin, Neoapigenin, Verbascoside, Oleuperin, Ligtroside, Cyanidin, Neocyanidin, p-Cumaric acid, Caffeic acid, Ferulic acid, Syringic, Gallic acid, Quercetin. These unique mixture of ingredients is obtained by a proprietary technique developed by R&AS (Siderno, Italy), termed by us Natural Molecular Imprinting (NMI<sup>TM</sup>), which has no environmental impact (zero pollution) because it makes use of natural polymers which selectively recognize target molecules not otherwise obtainable from water medium.<sup>2</sup>

## PROPERTIES

The polyphenols resulting from olives vegetation water have demonstrated an important hypotensive action. This latter is due to a peripheral and strong vasodilatation, related to the relaxation of smooth muscle in the arterial blood vessels and to a reduction in the capacity of contraction of smooth muscle cells of the vascular wall.

Moreover, a concomitant reduction of copper levels in the blood is observed, and because the enzymes that produce catecholamine need copper to function properly, the result is a reduction in the production of catecholamine, which are substances that increase blood pressure.

**Antiradical action.** The bio-polyphenols from olive fight various reactive oxygen species and also counter act the damage caused by free radicals to cells (lipid peroxidation). It is known that LDL exposed to free radicals, undergoes to an oxidative process, and this is the mechanism that triggers the evolution of atherosclerosis. Other studies suggest that antioxidants present in olives might have, thanks to these



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activities, vase- and –cardio protective action in humans. Some epidemiological studies suggest, in fact, that the Mediterranean diet is associated with a reduced incidence of cardiovascular diseases.<sup>3,4</sup> Recently it was shown, in more than 3000 individuals without clinical evidence of cardiovascular disease, that the high total antioxidant capacity of plasma and the low level of oxidation of low density lipoprotein (LDL) are associated with an increasingly growing consensus for the Mediterranean diet.<sup>5</sup> Compared with a diet rich in saturated fats, the Mediterranean diet, rich in oleic acid, is also associated with a lowering of LDL and triglycerides total with the maintenance of high-density lipoprotein<sup>6,7,8,9,10</sup> and a lowering of blood pressure<sup>11,12</sup>. In addition, polyphenols have been shown to be effective in preventing some forms of cancer,<sup>13,14,15</sup> and provided with anti-inflammatory<sup>16</sup> and antimicrobial activity.<sup>17,18</sup>

### **APPLICATIONS**

Use as nutraceutical and cosmeceutical in food, food supplements and cosmetic preparations. Other applications are as preservative in all fields in which antioxidant and antimicrobial properties of these extracts can be useful in preservation.

### **TOXICITY AND SIDE EFFECTS**

At present, no significant side effects or toxicity related to olive polyphenol ingestion or topic application has been reported. However, for precautionary purposes, medical advice is suggested during pregnancy and lactation.

*This informative note has been written with the scientific supervision of Stefano Manfredini, Professor of Pharmaceutical Chemistry and Toxicology at the Faculty of Pharmacy of the University of Ferrara. The partial or total reproduction of the text and images is prohibited without a prior written permission of AMBROSIALAB. While the information contained in the following note, are presented in good faith and believed to be accurate on the base of the references cited, they are provided for guidance only. Because many factors may affect processing or application/use, we recommend that you make test to determine the suitability of the product for your particular purpose prior to use.*



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### SUMMARY DATA SHEET

Aspect	Green to brown powder with metal reflex
Odor	Characteristic fresh olive oil odor
Total Plate Count (cfu/g)	<500
Coliforms	negative
Fungi (cfu/g)	< 10
Heavy metal (ppm)	< 10
As (ppm)	< 1
Solubility	Soluble in water and water/ethanol 1/1
Antioxidant activity	ORAC > 20.000 micromol TE/g



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### CERTIFICATE OF ANALYSIS

Name of the product: Ageraopherols™  
Botanical name: Olea europea  
Used part: drupes

INFORMATIONS	CHARACTERISTICS	RESULTS	ASSAY
Aspect	Crystalline solid, green-brown with metal reflex	√	visual
Odour and taste	Olive odor and taste	√	organolectic
Identification	Biophenols	√	HPLC
Extraction method	NMI-Ethanol selective extraction	√	
Vector	None	√	

#### Physical analysis:

INFORMATIONS	CHARACTERISTICS	RESULTS	ASSAY
Humidity	<5%	√	Karl Fisher
Solubility	Water	Full soluble	
Solubility	ethanol	Poor soluble	
Solubility	Water:ethanol 1:1	Soluble	
Solubility	ether	Insoluble	
Solubility	chloroform	Insoluble	
pH at 20°C		3.0-5.0	



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### Chemical analysis

INFORMATIONS	CHARACTERISTICS	RESULTS	ASSAY
Titre in glycosylated biophenols	>98%	√	HPLC
Heavy metal	<10 ppm	√	AAS
Arsenic	<1 ppm	√	AAS
Ash	<0.1%	√	
Lead	<0.1 ppm	√	
Cadmium	<0.1 ppm	√	
Mercury	<0.1 ppm	√	
Chrome	<1 ppm	√	
Organic acids	<1%	√	HPLC
Free sugars	<1%	√	HPLC
Ethanol	<50 ppm	√	

### Microbiology

INFORMATIONS	CHARACTERISTICS	RESULTS
Total Plate Count	<500 cfu/g	√
Yeasts and molds	<10 cfu/g	√
E. Coli	none	√
Salmonella	none	√
P. Aeruginosa	none	√
S. Aureus	none	√

Store in a fresh, dry place

keeping protected from light.



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