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Bioactive compounds from extra virgin olive oils: Correlation between phenolic content and oxidative stress cell protection

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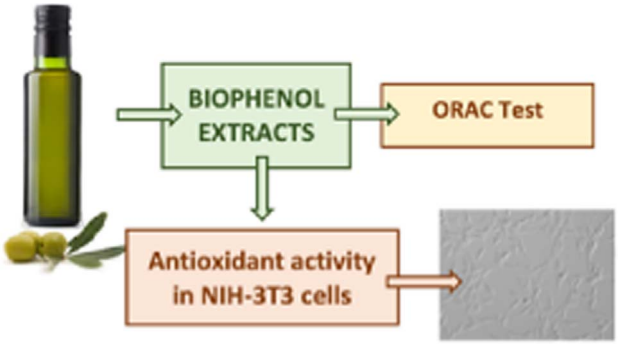
HIGHLIGHTS

- Mediterranean Olive Oils as powerful source of biophenols
- Complete analysis of the biophenol fraction
- Correlation between ORAC value and chemical classes of antioxidant species
- Relationship between molecular species and oxidative stress cell protection

GRAPHICAL ABSTRACT

Mediterranean Olive Oils are used as sources of biophenols. Results correlate molecular species and oxidative stress cell protection.



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ABSTRACT

When compared with other edible vegetable oils, the extra virgin olive oil (EVOO) exhibits excellent nutritional properties due to the presence of biophenolic compounds. Although they constitute only a very small amount of the unsaponifiable fraction of EVOO, biophenols strongly contribute to the sensorial properties of this precious food conferring it, for example, the bitter or pungent taste. Furthermore, it has been found that biophenols possess beneficial effects against many human pathologies such as oxidative stress, inflammation, cardiovascular diseases, cancer and aging-related illness. In the present work, the biophenolic content of 51 Italian and Spanish EVOOs was qualitatively and quantitatively identified and their antioxidant ability analyzed by oxygen radical absorbance capacity (ORAC) assay. Results indicated that the maximum relationship can be found if the ORAC value is correlated with the concentration of the large family composed by ligstroside and oleuropein derivatives together with their degradation products, hydroxytyrosol and tyrosol. Then, selected biophenolic extracts were tested in NIH-3T3 cell line to verify their ability in the recovery of the oxidative stress revealed by DCFH-DA

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assay. Results were linearly correlated with the concentration of ligstroside aglycone (aldehyde and hydroxyl form).

1. Introduction

Oxidative stress is one of major cellular features in the onset of many human pathological conditions such as Alzheimer's [1] and Parkinson diseases [2], renal disease [3], diabetes [4], ischemia [5], atherosclerosis [6], pulmonary dysfunction [7], cancer [8], and aging [9], and it occurs when excessive generation of free radicals produced during the normal cell metabolic processes is unbalanced by the antioxidant defense system. The latter includes endogenous enzymes (e.g. superoxide dismutase, catalase, glutathione reductase, glutathione (GSH) and glutathione peroxidase), metal binding proteins (ferritin, ceruloplasmin, lactoferrin and albumin) and exogenous chemical compounds mainly derived from fruits and vegetables (e.g. citrulline, taurine, creatine, selenium, zinc, A, C and E vitamins, and phenols).

Free radicals, i.e. reactive oxygen species (ROS) and reactive nitrogen species (RNS), are generated by unstable and highly reactive oxygen- and nitrogen-based molecules with unpaired electrons. Generally, oxidative damage to the cellular components results in alteration of the membrane properties such as fluidity and ion transport, enzyme activities and protein cross-linking [10]. Furthermore, damages can extend to nuclear component and cellular organelles [11].

Under physiological conditions, free radicals are normally produced during the cellular metabolism, the main source being the mitochondrion and, in particular, the electron transport chain (ETC) where the continuous movement of high-energy electrons can generate superoxides after interaction with O_2 molecules. In addition, other external factors can increase the levels of free radicals such as UV-light [12], air pollution [13], ionizing radiations [14,15] and smoking [16].

Due to the unhealthy consequences of oxidative stress and its implications, a huge number of studies have been devoted to the investigations of the therapeutic administration of antioxidants. They are chemical compounds, exclusively present in fruit and vegetables, able to prevent, slowing or terminating the radical formation reactions. The antioxidant redox effect is exerted against oxidation of lipids and proteins [17], and nucleic acids [18].

Thus, the interest of the researchers has been devoted to the discovery and valorization of food containing a large variety of antioxidants. Among these, extra virgin olive oil (EVOO) deserves a special place. EVOO is a key component of the traditional Mediterranean diet, which is believed to be associated with a relatively long life in good health.

Extra-virgin olive oil (EVOO) is recognized as one of the best food [19] for its capacity to prevent some diseases, such as cancer [20] and cardiovascular diseases [21], and to reduce their incidence in the western population. Furthermore, numerous studies show that olive oil reduces cholesterol [22], lowers blood pressure [22] and inhibits platelet aggregation [23]. EVOO is a complex mixture composed for about 98% by fatty acids esterified with glycerol, as mono-, di- and, prevalently, triglycerides, and unsaponifiable substances for the remaining 2%. This unsaponifiable fraction is constituted by squalene (about 50%), sterols, terpenic and aliphatic alcohols, methylsterols, biophenols and other compounds responsible for the oil particular taste and aroma [24].

Biophenols are responsible of the shelf-life of EVOO, because they avoid lipid oxidation through a variety of mechanisms based on radical scavenging, hydrogen atom transfer and metal-chelation [25]. Despite their low concentrations, phenolic compounds have been related with the healthy beneficial effects derived from consuming EVOO [26].

EVOO is unique among other vegetable oils because of the high levels of valuable biophenolic compounds, to which, together with the high content of unsaturated fatty acids, the healthy benefits of EVOO are attributed. The biophenolic fraction of EVOO consists of a heterogeneous mixture of compounds, each of them influencing the chemical properties and quality of EVOO.

In the present work, the phenolic fraction of 51 Sicilian EVOO samples was examined and the individual components were qualitatively and quantitatively determined. Moreover, the antioxidant efficacy of the 51 phenolic fractions was tested by the oxygen radical absorbance capacity (ORAC) method. Correlations between the concentration of the individual bioactive compounds or groups of compounds and ORAC values were studied. The highest correlation value was found with the large group composed by ligstroside and oleuropein derivatives, hydroxytyrosol and tyrosol. On the basis of this result, 9 biophenol extracts were chosen and evaluated for their ability to reduce ROS level induced in a cell model system by DCFH-DA assay.

2. Materials and methods

2.1. Extra virgin olive oil samples

32 Italian olive oil samples from Trapani geographical area (Trapani, Sicily) and 19 Spanish olive oil samples were analyzed. The Italian samples were a blend of the *Nocellara del Belice*, *Biancolilla* and *Cerasuola* cultivars while the Spanish ones were monocultivar samples of *Arbequina* and *Picual* olives. All the EVOOs were PDO (Protected Designation of Origin) registered, produced in 2014.

2.2. Chemicals and reagents

Folin-Ciocalteu, sodium carbonate, potassium persulfate ($K_2S_2O_8$), fluorescein, 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), gallic acid, Dulbecco's Modified Eagle's Medium (DMEM), Bovine Calf Serum, Penicillin-Streptomycin Solution (10,000 U/mL and 10,000 μg/mL, respectively), 2′,7′-Dichlorofluorescin diacetate (DCFH-DA), Luperox® TBH70X tert-Butyl hydroperoxide solution (TBH) were purchased from Sigma-Aldrich (Milan, Italia). CellTiter 96® Aqueous One Solution Assay (MTS) was purchased from Promega. The water used in all experiments was Millipore MilliQ.

2.3. Biophenol extract preparation

The biophenol extraction was based on the standard method for the determination of biophenols in olive oils of the International Olive Council [27] with slight modifications. 2 g of olive oil were used and 5 mL of methanol:water (80:20 v/v) solution were added. The mixture was put into vortex for 2 min and in ultrasonic bath for 25 min, then was centrifuged at 5000 rpm for 25 min at 20 °C in a Beckman Avanti 30 Compact centrifuge (Beckman Coulter, Italy). All extracts were stored in the dark at 4 °C.

2.4. Folin-Ciocalteu colorimetric assay

After retrieving the polar part, the determination of biophenols was done by using the Folin-Ciocalteu colorimetric assay. This method was performed accordingly to Hrncirik et al. [28] with minor modifications. An aliquot (0.2 mL) of the methanolic phase was diluted with water to a total volume of 5 mL, followed by the addition of 0.5 mL Folin-Ciocalteu reagent. After 3 min, 1 mL sodium carbonate solution (20% w/v) was added to the reaction mixture which was finally mixed and diluted with water to 10 mL. The absorbance of the solution was measured after 2 h against a blank sample on a Shimadzu Spectrophotometer at the

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