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Characterization of extra virgin olive oils produced with typical Italian varieties by their phenolic profile

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ABSTRACT

Evaluation of phenolic profile, antioxidant power, and protective capacity against oxidation of red blood cells (RBCs) of olive oil phenolic extracts (OOPEs) from several Italian varieties were studied. Phenolic profiles, and quantification of seven selected bioactive compounds were performed by RP-HPLC. OOPEs exhibited high antioxidant activity, and this capacity was positively related to their phenolic amount. In particular, OOPE5 (cv Gentile di Larino, Molise region) displayed the highest phenolic and *ortho*-diphenolic content as well as the strongest scavenging activity determined using 2,2′-diphenyl-1-picrylhydrazyl (DPPH·) (87% DPPH· inhibition). Protective capacity against stressed RBCs was investigated through the evaluation of methemoglobin (MetHb) and malondialdehyde (MDA) levels. OOPE5 was the most active against methemoglobin production (53.7% reduction), whereas OOPE1 (cv Lavagnina, Liguria region) showed the highest protection toward malondialdehyde (83.3% reduction). Overall the selected oils showed qualitative and quantitative differences in phenol composition, and this variability influenced their protective effect against oxidative damages.

1. Introduction

The importance of the Mediterranean diet is well-recognized by now, and a wide number of epidemiological studies have demonstrated that the incidence of coronary heart disease and some types of cancer is lower in the Mediterranean area (Alarcon de la Lastra, Barranco, Motilova, & Herrerias, 2001).

Several investigations have established that olive oil, which is the principal source of fats in the Mediterranean basin, has positive effects on human health, in particular to prevent breast and colon cancers, and as regards diabetes accompanied by hypertriacylglycerolaemia, inflammatory and autoimmune diseases such as rheumatoid arthritis (Visioli, Poli, & Galli, 2001). The beneficial properties of olive oil can be attributed to its high content of monounsaturated fatty acids; in particular, oleic acid (18:1, ω-9) reduces plasma levels of LDL-cholesterol and increases those of HDL-cholesterol. Olive oil has also a positive effect on blood pressure, and its consumption fosters the increase of ω-3 fatty acids and the decrease of ω-6 fatty acids in the erythrocyte membrane. This effect seems to be responsible for the lowering of the systolic and diastolic pressure in patients subjected to a diet with olive oil, compared to patients fed with a diet containing sunflower oil (Herrera, Pérez-Guerrero, Marhuenda, & Ruiz-Gutiérrez, 2001).

Numerous studies have established that the olive oil non-glyceride fraction rich in phenolic compounds, which play a part in the determination of the organoleptic features of this product (Servili et al., 2009), also contributes to the benefits on human health (Tripoli et al., 2005). It is known that the healthy properties of the phenolic compounds are correlated to their antioxidant activity (Papadopoulos & Boskou, 1991); in fact, these molecules have a positive effect on cardiovascular diseases, being able to reduce the oxidation of LDL due to their ability to scavenge superoxide radicals which are involved in the pathogenesis of atherosclerosis (Visioli, Bellomo, & Galli, 1998). Through their ability to react with the reactive oxygen and nitrogen species (ROS and RNS), phenolic compounds also concur to the prevention of some types of cancer. Among these molecules, *ortho*-diphenols have been demonstrated to considerably contribute to the oxidation stability of the olive oil, and hydroxytyrosol, also known as 3,4-dihydroxyphenylethanol (3,4-DHPEA), represents the major *ortho*-diphenol present in virgin olive oil, in both free and esterified form (oleuropein aglycone). This compound is known for its capacity to inhibit platelet

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aggregation, to neutralize free radical-induced cytotoxicity in human intestinal epithelial cells *in vitro*, and for its antimicrobial activity (Hu, He, Jiang, & Xu, 2014).

The Mediterranean area contributes to more than 95% of the olive oil worldwide production, with 75% coming from European Union countries, primarily Italy, Spain and Greece. In Italy olive oil production is mainly distributed in several regions of the central and southern areas; however, in recent years, this activity has been increasing in the northern regions with temperate climate. As extra virgin olive oil is pressed fruit juice without additives, the factors influencing its quality and taste include cultivar (Kalua, Allen, Bedgood, Bishop, & Prenzler, 2005), technological aspects of productive process (Servili et al., 2004), and pedoclimatic conditions of olive growth (Dabbou et al., 2010). As consequence, the phenolic content differs from a qualitative and quantitative point of view depending on several factors, both agronomic and technological with a concentration of these compounds in extra virgin olive oil ranging from 50 to 800 mg/kg (Tripoli et al., 2005). Pérez et al. (2014) observed that there was a large variability, in terms of phenolic content, in the progeny derived by crossing two pure olive cultivars, demonstrating that also genetic variability plays a fundamental role in the phenolic pattern.

The demand for olive oils with well-defined organoleptic, nutritional and commercial characteristics has recently re-evaluated the function of the cultivar as an important factor in the qualification of Italian extra virgin olive oil production. The safeguard of several qualitative characteristics of the olive oil goes through the conservation and valorisation of the varieties of a specific territory; in this way, it is possible to protect those characteristics that define the "uniqueness" of an olive oil.

Human erythrocytes (red blood cells, RBCs) represent a metabolically simplified model system useful in the evaluation of antioxidant properties of many compounds, because these cells are particularly exposed to oxidative hazard due to their specific role as oxygen carriers (Arbos, Claro, Borges, Santos, & Weffort-Santos, 2008; Manna et al., 2002; Paiva-Martins et al., 2009). RBCs are continuously exposed to oxidant agents from different exogenous and endogenous sources (in particular, ROS) which are neutralized by several endogenous antioxidants. However, if a situation of impairment of antioxidant defenses and/or accumulation of ROS occurs, an "oxidative stress" condition develops, inducing oxidative damages to RBCs constituents. In particular, the formation of methemoglobin (MetHb), unable to bind and carry oxygen is provoked, and lipid peroxidation with generation of unwanted products, the main of which is the malondialdehyde (MDA) is stimulated (Pandey & Rizvi, 2011).

The aim of the present work was to characterize twelve extra virgin olive oils from several regions of the Italian peninsula, produced by different cultivars, and manufacturing techniques, through the estimation of their total phenolic and *ortho*-diphenolic content, as well as the evaluation of free-radical scavenging activity of their phenolic extracts. In addition, the amount of important phenolic molecules such as: oleuropein, hydroxytyrosol, tyrosol (known as *p*-hydroxyphenylethanol; *p*-HPEA), *ortho*-coumaric, *para*-coumaric, caffeic, and protocatechuic acids was also determined. The capacity of the phenolic extracts to protect RBCs from *tert*-butyl hydroperoxide (*t*-BHP) induced oxidative injury was also investigated. More in detail, the ability of the different extracts to reduce MetHb and MDA production was compared.

2. Materials and methods

2.1. Chemicals

Gallic acid, caffeic acid (CAA), Folin–Ciocalteu reagent, 2,2′-diphenyl-1-picrylhydrazyl (DPPH·), *tert*-butyl hydroperoxide (*t*-BHP), oleuropein (OP), tyrosol (TY), hydroxytyrosol (HTY), protocatechuic acid (PCA), *para*- and *ortho*-cumaric acids (*p*-CA and *o*-CA), dinitrophenylhydrazine (DNPH), *tert*-butyl hydroperoxide (*t*-BHP) and 1,1,3,3-tetraethoxypropane (TEP) were purchased from Sigma Chemical Co. (St. Louis, MO). Methanol, hydrochloric acid (HCl), *n*-hexane, and HPLC grade acetonitrile were obtained from Romil Ltd (Cambridge, UK). All other reagents were analytical grade.

2.2. Extra virgin olive oils sampling

Twelve extra virgin olive oils, produced by local oil mills from three regions representatives of northern, central and southern Italy, were chosen for the analyses. Characteristics (geographical regions, olive varieties, altitude and harvest date) of the olive oil samples are summarized in Table 1. All oil samples were kept at room temperature in dark bottles until analyses.

2.3. Extraction and analysis of phenolic compounds

The phenolic extracts from the extra virgin olive oils (olive oil phenolic extracts, OOPEs) were prepared according to Montedoro,

Table 1
Geographical olive varieties information and characterization of the twelve extra virgin olive oil extracts.

OOPE	Italian region	*Cultivar*	Altitude (m)	Harvest date	Total phenols (mg/kg)	*ortho* Diphenols (mg/kg)	*ortho* Diphenols/Total phenols (%)	DPPH· inhibition (%)
1	[a]LIGURIA	Lavagnina	300	November 2011	1030 ± 14	270 ± 19	26.21	76 ± 0.82
2	LIGURIA	Lavagnina	50	November 2011	290 ± 35	50 ± 3	17.24	70 ± 1.03
3	LIGURIA	Taggiasca	350	December 2011	700 ± 19	130 ± 4	18.57	76 ± 1.11
4	LIGURIA	Taggiasca	300	February 2012	490 ± 18	60 ± 3	12.24	74 ± 0.65
5	[b]MOLISE	Gentile di Larino	300	October 2011	2180 ± 69	670 ± 25	30.73	87 ± 1.45
6	MOLISE	Gentile di Larino	150	November 2011	320 ± 32	60 ± 2	18.75	73 ± 0.83
7	MOLISE	Leccino	580	November 2011	1520 ± 3	490 ± 18	32.24	79 ± 0.92
8	MOLISE	Leccino	250	November 2011	600 ± 29	230 ± 10	38.33	72 ± 1.21
9	[c]SICILY	Cerasuola	90	November 2011	1340 ± 33	410 ± 8	30.60	82 ± 1.14
10	SICILY	Cerasuola	170	November 2011	670 ± 44	140 ± 4	20.90	73 ± 0.61
11	SICILY	Biancolilla	200	November 2011	680 ± 27	130 ± 7	19.12	73 ± 1.64
12	SICILY	Biancolilla	300	November 2011	340 ± 7	100 ± 5	29.41	72 ± 1.21

All extra virgin olive oils were produced by using 100% of the cultivar indicated in the Table. The colorimetric assays were conducted in triplicate, by assaying three extracts obtained separately from each sample of olive oil. Data are mean ± SD values.

[a] Northern Italy.
[b] Central Italy.
[c] Southern Italy.