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Evaluation of contribution of micro and macro components to oxidative stability on virgin oils obtained from olives characterized by different health quality

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Title
Valutazione del contributo di micro e macro componenti alla stabilità ossidativa in oli vergini ottenuti da olive a diverso stato fitosanitario

Key words
Virgin olive oils, oxidative stability, phenols, fatty acids, olive quality

Parole chiave
Oli vergini di oliva, fenoli, stabilità ossidativa, stato fitosanitario delle olive

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Summary
The high oxidative stability of extra virgin olive oil is mainly due to its fatty acid composition, in particular to the high monounsaturated-to-polyunsaturated ratio, and to the presence of minor compounds having phenolic structure, that also have a major role in preventing oxidation. Several classes of phenolic compounds have been identified in extra virgin olive oil (phenolic acids, phenyl ethyl alcohols, flavonoids, lignans and secoiridoids) and among them the secoiridoids (aglyconic derivatives of oleuropein and ligstroside) are the most abundant constituents. Phenolic compounds belonging to *o*-diphenolic category, such as oleuropein aglycon (OA), decarboxymethyl-oleuropein aglycon (DOA), and hydroxytyrosol (HYTY), are mainly responsible for the oxidative resistance of extra virgin olive oil. In this work, results dealing with oxidative stability (OSI) of several samples (n=32) obtained from olives with a different degree of *Bactrocera oleae* attack (0-85%), are shown. Moreover an electrochemical evaluation of the antioxidant power (AOP) of the phenolic fraction has been carried out. This method enables the recognition of the phenols that may be easily oxidised. Data demonstrate an increase of products coming from hydrolytic and oxidative degradations of fatty acids as a function of the degree of olive fly attack. Moreover, their negative contribution to oil stability has been established. Olives soundness influenced mainly the phenolic content of samples, which as a result, affected the OSI values. Fatty acid composition was involved to a lesser extent.

Riassunto
L'elevata stabilità ossidativa dell'olio extra vergine di oliva è dovuta soprattutto alla sua composizione in acidi grassi, in particolare all'elevato rapporto monoinsaturi-polinsaturi ed alla presenza di composti minori a struttura fenolica che svolgono un ruolo principale nella prevenzione dell'ossidazione. Nell'olio extra vergine di oliva sono state identificate diverse classi di composti fenolici (acidi fenolici, alcoli fenil-etilici, flavonoidi, lignani e secoiridoidi) e fra queste i secoiridoidi (derivati agliconici di oleuropeina e ligstroside) sono i costituenti più abbondanti. I composti fenolici appartenenti alla categoria degli *o*-difenoli, come l'oleuropeina aglicone

(OA), la decarbossimetil-oleuropeina aglicone (DOA) e l'idrossitirosolo (HYTY) sono i maggiori responsabili della resistenza ossidativa dell'olio extra vergine di oliva. In questo lavoro sono presentati i risultati relativi alla stabilità ossidativa (OSI) di numerosi campioni prodotti da olive con un diverso grado d'infestazione da parte della mosca dell'olivo. E' stata condotta inoltre la valutazione del potere antiossidante (AOP) della frazione fenolica tramite un metodo elettrochimico diretto in grado di evidenziare i composti più facilmente ossidabili. La sperimentazione ha evidenziato un incremento dei prodotti della degradazione idrolitica ed ossidativa degli acidi grassi in funzione del grado di attacco della mosca dell'olivo ed un loro contributo negativo alla stabilità degli oli; inoltre lo stato fitosanitario delle olive è risultato influenzare maggiormente la dotazione in fenoli dei campioni, con un importante riflesso sui valori di OSI, rispetto alla composizione in acidi grassi.

Introduction

Shelf life of virgin olive oil depends strictly on the autoxidation of fatty acids that, in the form of triglycerides, diglycerides or in free form, are the major components of this product. The higher ratio between monounsaturated and polyunsaturated fatty acids of olive oil than other edible oils must be considered a very important stabilizing factor: in fact, the oxidation process moves quicklier depending on the number of double bonds of fatty acids, thus the linolenic acid is easier triggered than oleic acid (1). It is well known that is not possible to stop this process but only to slow it down. Several authors (2-4) demonstrated the effectiveness of some endogenous antioxidants to delay the oxidative process by preventing the propagation step of lipid peroxidation; in particular, phenolic molecules both lipophilic (as tocopherols) and hydrophilic can be able to enhancing the oxidative stability through a chain breaking mechanism.

According to Frankel (5) and more recently to Afri et al. (6), in the bulk oil system the hydrophilic phenols are more protective against oxidation than the lipophylic antioxidants due to their orientation in the air-oil or water-oil interface (whereas tocopherols remain solubilised in the oil). The extent of the antioxidant power of phenols is closely connected to their oxidation potentials and so to their ability to give electrons or hydrogen atoms to scavenge peroxyl and alkoxyl radicals. Several studies have demonstrated a positive linear relationship between oil stability and the total phenol content and, within this class, have evidenced a strongest activity of *o*-diphenols (7, 8).

In literature there are numerous studies about the relative antioxidant potency of the individual olive oil phenols, but the choice of the analytical method used to evaluate them can lead to different results sometimes conflicting (8). The assays based on the evaluation of the hydrogen-donating activity of molecules or extracts towards some free radicals, stable and having a specific intensive absorbance in the visible region (DPPH and ABTS), are widely used (9). Otherwise, accelerated methods (under forced air flow and high temperature) are generally employed to estimate the trends of the oxidative process in a relatively short period of time, using instrumentation as Rancimat and OSI. These indices, express in hours, are very useful because they

give a good estimation of the susceptibility of the oil to oxidative degeneration with relation to antioxidant and pro-oxidant components (10).The induction period, generally used as an index of resistance to oxidation, is expressed as the time required to reach the endpoint of oxidation corresponding to a sudden change in the rate of oxidation. It is important to evidence that the stability of virgin olive oil is improved due to synergistic interactions between the various antioxidants present (both phenolic and non-phenolic) and the lipid composition. The results obtained by Aparicio et al. (11) confirmed that phenols and oleic/linoleic ratio had together the maximum correlation with oxidative stability by Rancimat and their contribution reached to 78%.

Tests based on the electrochemical properties were recently applied (8, 12, 13). In 1999 Mannino et al. (12) proposed an electrochemical procedure, working directly on virgin olive oil diluted in organic solvent, as alternative to Rancimat and ABTS radical assay, to establish its antioxidant power. These are focalised on a simple concept: the oxidation potential of a compound depends on the energy required to donate an electron; the lower the oxidization potential, the more easily the compound will donate an electron, and the higher its expected antioxidant activity. Del Carlo et al. (13) on the basis of hydrodynamic voltammetry experiments chose quercetin as standard molecule to calibrate the method at potential 0 mV vs Ag/AgCl reference electrode (QE0).

Material and methods

Samples. Thirty-two virgin olive oils different for percentage (2-85%) of fly attack (*Bactrocera oleae*), variety of olives and technological system used (by pressure or centrifugation, with or without destining phase) were studied. The samples were from different industrial mills located in Abruzzo region (Italy).

Extraction of polar phenolic fraction. Phenolic compounds were extracted from virgin olive oil by a liquid-liquid extraction method according to Pirisi et al. (14).

Electrophoretic procedure. The separation by CE was carried out using the developed method proposed by Carrasco-Pancorbo et al. (15).

Evaluation of oxidative stability under forced conditions. These analyses were carried out in an eight-channel oxidative stability instrument (OSI). Virgin olive oil samples were heated at 110°C under atmospheric pressure, and air was allowed to bubble (20 L h^{-1}) through the oil. Under these conditions short-chain volatile acids are produced and they were recovered and measured conductimetrically in distilled water. The time (express as hour and hundredth of hour) required to produce a sudden increase in conductivity determines an induction period (OSI time).

Fatty acid composition. Fatty acid composition of samples was determined as FAMEs by capillary gas chromatography (GC) analysis after alkaline treatment, according to Bendini et al (4);

Acidity and peroxide value. These parameters were determined according to the official methods described in European Regulation EEC 2568/91 and the following amendments (16).

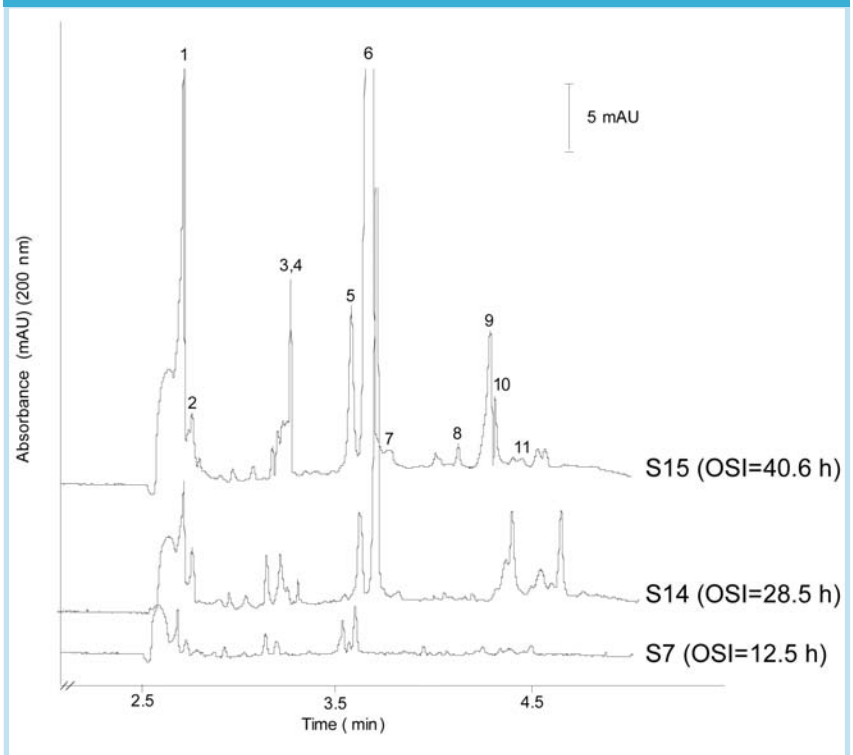
Antioxidant power determination. Phenolic extracts were measured in a FIA apparatus at a potential set at 0 mV vs Ag/AgCl. The flow rate of phosphate buffer (pH 7.4) was 150 µl min^{-1}. All extracts were injected in triplicate. The current produced during the electrochemical oxidation of the phenolic compounds was recorded (13).

Results and discussion

It is known that the antioxidant efficiency of some natural compounds is very dependent on structural factors such as number and position of hydroxyl groups in the molecule. The components having at least one *o*-catechol group, generally named *o*-diphenols, are characterized by both higher ability to delocalise the unpaired electrons and better capacity to form intra-

molecular hydrogen bonds than other phenols. The major *o*-diphenols of virgin olive oil, under a quantitative point of view, belong to secoiridoid class and in particular are oleuropein derivatives. These compounds exist in different isomeric forms in virgin olive oil (17) and can be identified and quantified separately depending on the analytical technique used (18). In this work, by the setting up electrophoretic method, eleven peaks were eluted within five minutes as can be seen in figure 1. The principal components of analysed samples were *o*-diphenols as isomeric forms of decarboxy-methyl-oleuropein (DOA) and oleuropein (OA), ranging within a very large interval of values (Table 1). These compounds oxidized easier than other phenols when the oil was under oxidative stress. Because of that they could be considered as more effective antioxidants. In fact, the statistical analysis of the data revealed a good correlation between *o*-diphenols and oxidative stability valued by OSI ($r=0.86$, $p<0.001$). According to Aparicio et al. (11), phenols and particularly *o*-diphenols influenced the oxidative stability (in our study valued by OSI test) of oil samples stronger than the ratio between oleic acid and linoleic acid ($r=0.57$, $p<0.001$). Since the electrochemical behaviour of phenols depends on their structural features, it can provide a chemical basis for describing their

Figure 1 - Capillary zone electropherograms of phenolic extract of three samples (S7, S14 and S15). Peak identification numbers: 1, LIG (a); 2, TY; 3, PIN; 4, AcPIN; 5, OA (a) + DOA (a); 6, DOA (b); 7, OA (b); 8, EA (a); 9, OA (c) + LIG (c) + DOA (c) + EA (b, c); 10, HYTY; 11, DOA (d) + EA (d).



reactivity as electron donors and thus their antioxidant functionality. Electrochemical procedure provided the QE0 values of virgin olive oil extracts which fit significatively with *o*-diphenols ($r=0.59$, $p<0.001$) but especially with content of an isomeric form of OA and DOA ($r=0.68$, $p<0.001$). This electrochemical experimentation confirmed that oleuropein aglycon and decarboxymethyl-oleuropein aglycon are the strongest antioxidant molecules of virgin olive oil, whereas a lower influence was evidenced for hydroxytyrosol ($r=0.45$, $p<0.001$). Statistical data confirmed also that pinoresinol and 1-(+)-acetoxypinoresinol, two molecules belonging to lignan class (19), are not able to explicate an efficacious antioxidant activity, as evidenced in precedent experimentations (8). It is noteworthy that the degree of fly attack resulted very related to percentage of free acidity ($r=0.77$, $p<0.001$) and with the minor extent ($r=0.58$, $p<0.001$) to peroxide

Table 1 - The mean and the range of the chemical variables analysed in the virgin olive oil samples. %ATT, percentage of olives attacked by Bactrocera oleae (from 2% to 85%); FA, free acidity express as g of oleic acid on 100 g of oil; PV, peroxide value express as meq O_2 kg^{-1} oil; OSI, express in hour and hundredth of hour; O/L, oleic to linoleic ratio; QE0, antioxidant activity value express as quercetin equivalent; TP, total phenols as sum of all molecules identified and quantified by CZE; *o*-DIPH, sum of OA(a) + DOA(a), DOA(b), OA(b) and HYTY; LIG (a, c), isomeric forms of ligstroside aglycon; TY, tyrosol; PIN+AcPIN, pinoresinol+1-(+)-acetoxypinoresinol; EA (a-d), isomeric forms of elenolic acid; OA(a-c) isomeric forms of oleuropein aglycon; DOA(a-d), isomeric forms of decarboxy-methyl-oleuropein; HYTY, hydroxytyrosol. Simple phenols have been quantified as mg of 3,4-dihydroxyphenylacetic acid kg^{-1} of oil. Secoiridoids and lignans have been quantified as mg of oleuropein glucoside kg^{-1} of oil. *Pearson's correlations with $p<0.001$, for 32 samples.

Mean and range values of chemical parameters				*Correlations**		
Parameter	*Mean*	*Min*	*Max*	*OSI*	*QE0*	*%ATT*
OSI	22.28	7.50	42.05	-	-	*-0.54*
QE0	43.07	5.19	167.01	-	-	-
FA	0.72	0.14	3.81	*-0.59*	-	*0.77*
PV	9.9	5.3	19.0	*-0.62*	-	*0.58*
O/L	9.99	4.17	14.32	*0.57*	-	-
TP	100.8	15.4	279.5	*0.79*	*0.69*	*-0.52*
o-DIPH	70.94	8.44	220.83	*0.86*	*0.59*	*-0.50*
Peak 1: LIG(a)	16.76	4.02	46.04	*0.58*	*0.56*	*-0.47*
Peak 2: TY	1.57	0.95	2.73	-	*0.52*	-
Peak 3+4: PIN+AcPIN	11.78	2.33	29.49	-	-	-
Peak 5: OA(a) + DOA(a)	10.56	2.55	21.66	*0.74*	*0.68*	*-0.53*
Peak 6: DOA(b)	39.16	1.39	158.98	*0.86*	*0.51*	-
Peak 7: OA(b)	4.11	nd	16.78	-	*0.67*	-
Peak 8: EA(a)	1.71	nd	5.67	*0.57*	*0.73*	*-0.50*
Peak 9: OA(c)+LIG(c)+DOA(c)+EA(b,c)	10.83	nd	48.51	*0.73*	-	-
Peak 10: HYTY	1.69	nd	4.17	-	*0.45*	*-0.46*
Peak 11: DOA(d)+EA(d)	4.74	nd	12.34	*0.59*	-	-

value; these last correlations evidenced as this dangerous physiopathology leads above all to the hydrolytic degradations of lipid due to a breaking up of the wall cells. The increases of free acidity and peroxides negatively affect the oxidative stability of virgin olive oils, as demonstrated the negative correlation values reported in table 1.

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