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Influence of heating on antioxidant activity and the chemical composition of some spice essential oils

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Abstract

Oxidation of lipids is one of the basic processes causing rancidity in food products. Since application of natural antioxidants may be one of the technically simplest ways of reducing fat oxidation, we studied the effect of heating on antioxidant effectiveness and the chemical composition of basil, cinnamon, clove, nutmeg, oregano and thyme essential oils. When maintained at room temperature, all the oils tested appeared endowed with good radical-scavenger properties in the DPPH· assay (effectiveness order: clove ≫ cinnamon > nutmeg > basil ⩾ oregano ≫ thyme). When heated up to 180 °C, nutmeg oil (but not the other essential oils under study) showed a significantly higher free radical-scavenger activity and evident changes in its chemical composition. Furthermore, the ability of these essential oils to protect α-tocopherol, contained in virgin olive oil, against thermal oxidative degradation was investigated. All the essential oils tested appeared able to prevent α-tocopherol loss following oil heating at 180 °C for 10 min (efficiency order: clove > thyme ⩾ cinnamon > basil ≫ oregano > nutmeg). In conclusion, the essential oils under study exhibited good antioxidant properties and might be efficiently used to control lipid oxidation during food processing.
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Keywords: Essential oil; Antioxidant; Heating; α-Tocopherol; Food processing

1. Introduction

Oxidation of lipids, which occurs during raw material storage, processing, heat treatment and further storage of final products, is one of the basic processes causing rancidity in food products, leading to their deterioration (Donelli & Robinson, 1995). Furthermore, products of lipid oxidation may influence other food constituents. Oxidative deterioration can result in alterations of organoleptic characteristics, e.g., taste and aroma, in the finished products, making them unacceptable to the consumer. In addition, oxidised lipids may have undesiderable effects on the human organism (Benzie, 1996).

Thus, due to health protection and economic reasons, many investigations have been undertaken with the aim to enhance the stability of lipids and lipid-containing products.

Application of antioxidants is one of the technically simplest ways of reducing fat oxidation (Frankel, 1993; Karpińska, Borowski, & Danowska-Oziewicz, 2001). Recently, there has been a certain reluctance to use synthetic additives, including antioxidants, for food products. In particular, some synthetic antioxidants, such as BHT and BHA, might be dangerous for living organisms (Attmann, Grunov, Mohr, Richterreichhelm, & Wester, 1986; Powell, Connelly, Jones, Grasso, & Bridges, 1986).

There is an increasing interest in herbs and spices as sources of natural antioxidants (Baratta et al., 1998; Dorman, Deans, & Noble, 1995; Lis-Balchin, Deans, & Eaglesham, 1998). Especially worthy of note are spices and herbs used for many years to enhance the sensory features of food.

However, in the literature, there are no data about the effects of food processing procedures (including thermal treatment) on the antioxidant activity or the chemical composition of spice essential oils employed in the food industry. Thus the objective of this study was to

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study the effect of heating on free radical-scavenger effectiveness and on the chemical composition of some spice essential oils (basil, cinnamon, clove, nutmeg, oregano, thyme). In addition, we have investigated their potential application to protect α-tocopherol contained in virgin olive oil against thermal oxidative degradation; in fact, olive oil may be a good substrate for evaluating the antioxidant activity of ingredients employed in food processing.

2. Materials and methods

2.1. Materials

Methanol (analytical grade) was purchased from Carlo Erba Reagenti (Milan, Italy), (±)-α-tocopherol and 2,2-diphenyl-1-picrylhydrazyl radical from Sigma–Aldrich (Milan, Italy), eugenol from Extrasynthese (Lyon, France), *n*-hexane and isopropyl alcohol (HPLC grade) from Mallinckrodt Baker (Milan, Italy). The essential oils of *Cinnamomum zeylanicum* Nees. (cinnamon), *Myristica fragrans* Houtt. (nutmeg) and *Thymus vulgaris* L. (thyme) were purchased from Adrian S.A. (Marseille, France); the essential oils of *Eugenia caryophyllata* Thunb. (clove), *Ocimum basilicum* L. (basil) and *Origanum floribundum* Mumby (oregano) were from Aboca (Sansepolcro, Arezzo, Italy). The following authentic compounds were employed as standards in gas-chromatography analyses: acetyl-eugenol, camphene, carvacrol, β-caryophyllene, caryophyllene oxide, 1,8-cineole, cinnamic aldehyde, *p*-cymene, eugenol, α-humulene, (+)-limonene, linalool, myrcene, myristicin, α-phellandrene, β-phellandrene, α-pinene, β-pinene, sabinene, α-terpinene, γ-terpinene, terpinen-4-ol and thymol (Extrasynthese, Lion, France) and safrole (Roth, Karlsruhe, Germany).

Membrane filters (0.45 μm) were from Waters-Millipore Corporation (Milford, MA). Extravirgin olive oil were purchased from a local supermarket.

2.2. Methods

2.2.1. Measurement of the free radical-scavenging activity and chemical analysis of essential oils after exposure to different temperatures

2.2.1.1. Sample preparation. Samples (300 mg) of each essential oil or of eugenol (as a positive control to check the technical procedures used in this experimental model) were put in a glass tube with screw cap and kept for 3 h at room temperature or incubated at different temperatures (80, 100, 120 and 180 °C). At the end of the incubation, the samples were cooled in an ice bath and immediately used to determine their radical-scavenging activities or their chemical compositions. All experiments were carried out in triplicate.

2.2.1.2. Bleaching of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH test). The antiradical activity of essential oils was determined by using the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH·) (Rapisarda et al., 1999; Saija et al., 1998). In its radical form, DPPH· has an absorption band at 515 nm which disappears upon reduction by an antiradical compound.

Briefly, the essential oils (maintained at room temperature or exposed to different temperatures as described in Section 2.2.1.1) were appropriately diluted with methanol, then an aliquot (20 μl) was added to 1.5 ml of daily prepared DPPH· solution (0.025 g/l in methanol); an equal volume (20 μl) of the vehicle alone (methanol) was added to control tubes. After 20 min the absorbance at 515 nm was measured by means of a Shimadzu UV-1601 UV–visible spectrophotometer. All determinations were carried out in duplicate.

The remaining DPPH· concentration ($[DPPH\cdot]_r$) was calculated from the following calibration curve, determined by linear regression

$$A_{515} = 2935.68[DPPH\cdot]_r - 2.18 \times 10^{-3},$$

where $[DPPH\cdot]_r$ was expressed as g/l and correlation coefficient (r) = 0.999.

The percentage of $[DPPH\cdot]_r$ (% $DPPH^{\cdot}_r$) was calculated as follows

$$\%\ DPPH^{\cdot}_r = \{[DPPH\cdot]_r/[DPPH\cdot]_0\} \times 100,$$

where $[DPPH\cdot]_0$ was the DPPH· concentration at the beginning of the experiment.

2.2.1.3. GC analysis of essential oils after exposure to different temperatures. The essential oils (maintained at room temperature or exposed to different temperatures as described in Section 2.2.1.1) were analysed by gas–liquid chromatography (GC). A gas–liquid chromatograph Model HRGC 5300 Mega Series (Carlo Erba, Milan, Italy) equipped with a flame ionization detector and with a ThermoQuest data processor "chrom-card" for integration of peak areas. Before injection, 100 μl of essential oil were diluted to 1 ml with acetone; then an aliquot (1 μl) of the solution was injected into the GC apparatus. Chromatography was performed using a glass capillary column Supelcowax 10 (60 m long, 0.25 mm i.d., 0.25 μm film thickness; Supelco Inc., Bellefonte, Palo Alto, CA, USA). The injector and the detector were maintained at 250 °C; helium was used as carrier gas. The split ratio was 1:70 for basil, cinnamon and clove, and 1:50 for nutmeg, oregano and thyme.

The other analytical conditions were as follows:

Basil, cinnamon, clove. Total analysis time: 55 min; temperature programming: 60 °C, as initial temperature, for 6 min, 5 °C/min to 120 °C, holding for 10 min, 4 °C/min to 180 °C.

Oregano and thyme. Total analysis time: 55 min; temperature programming: 60 °C, as initial temperature, for 4 min, 4 °C/min to 210 °C.

Nutmeg. Total analysis time: 100 min; temperature programming: 45 °C, as initial temperature, for 6 min, 3 °C/min to 220 °C.

All chemical analyses were carried out in duplicate. Volatile components were identified on the basis of gas-chromatographic retention times, determined by using a homologous series of C_7–C_{27} *n*-alkanes on an apolar column (AT-1, 60 m long, 0.25 mm i.d., 0.10 μm film thickness; Alltech Associates, Deerfield, IL) and a polar column (Supelcowax 10); furthermore, literature data and the retention time of authentic compounds previously analyzed were also used. The relative amount of each individual component of the essential oils, expressed as the percentage of the peak area relative to the total GC peak area, was computed on the Supelcowax 10 column without using correction factors.

2.2.2. Protective effect of essential oils on the decrease, induced by thermic stress, of α-tocopherol content in extravirgin olive oil

2.2.2.1. Sample preparation. Two hundred milligrammes of commercial extravirgin olive oil were put in a glass tube with a screw cap, and treated with 10 μl of essential oil or 5 μg of eugenol (as a positive control to check the technical procedures used in this experimental model); then the samples were heated at 180 °C for 10 min. At the end of the incubation, the samples were quickly cooled, diluted with *n*-hexane (800 μl) and stored at −70 °C until analysed. An oil sample, treated as above but maintained at room temperature, was employed as control to determine the basal α-tocopherol content. All experiments were carried out in triplicate.

2.2.2.2. HPLC analysis of α-tocopherol content. Olive oil α-tocopherol content was determined according to the method described by Psomiadou and Tsimidou (1998). An HPLC apparatus, consisting of a Varian 5000 HPLC pump (Varian Walnut Creek, CA) and equipped with a Rheodyne injection valve (model 7125), a 20 μl fixed loop (Rheodyne, Cotati, CA), an HP 1046A Hewlett-Packard programmable fluorescence detector (Hewlett-Packard GmbH, Waldbronn, Germany), and a 4290 Varian integrator, was employed for the analysis.

Olive oil samples were filtered prior to injection, using a Millex HV13 filter (0.45 μm; Waters-Millipore Corporatiopn, Milford, MA), and an aliquot (20 μl) of sample was injected (in duplicate) into the HPLC apparatus. Separation was achieved on a Lichrosorb SI-60 column (particle size: 5 μm; 250 mm × 4 mm i.d.; Supelco Inc., Bellefonte, PA). *n*-Hexane (A) and 2-propanol (B) were used as eluents. The gradient was 1% B for 10 min, 1–30% B in 20 min, 30–1% B in 20 min and 1% B for 6 min. The flow rate was set at 1 ml/min. Fluorescence detector was set at 290 nm (excitation) and 330 nm (emission).

α-Tocopherol was identified on the basis of the retention time of a known standard; quantification was achieved by using external α-tocopherol standards (prepared in triplicate) and calculating a standard curve (concentration vs. peak area) by linear regression analysis. All chemical analyses were carried out in duplicate.

2.2.3. Statistical analysis

Data were analyzed by the Student's *t*-test for paired data (free radical-scavenging activity and chemical analysis) or for unpaired data (protective effect against thermal stress-induced decrease of α-tocopherol contained in olive oil). A statistically different significance was accepted when $P < 0.05$.

3. Results and discussion

The identified components present with a concentration >5% in the analyzed essential oils (basil, cinnamon, clove, nutmeg, oregano and thyme) are listed in Table 1 and account for 74.1%, 79.6%, 98.2%, 77.1%, 65.6% and 77.6% of the oils under study, respectively. The major components in the essential oils examined were the following: the phenylpropanoids eugenol (82.6%) and cinnamic aldehyde (67.9%) in clove and cinnamon, respectively, the oxygenated monoterpenes linalool (53.3%), thymol (45.3%), carvacrol (48.9%) in basil, thyme and oregano, respectively, and the monoterpene hydrocarbons α-pinene (22.2%), β-pinene (15.1%) and sabinene (20.2%) in nutmeg. Several papers report that all these compounds, with the exception of linalool, possess significant antioxidant activity in several model systems (Dorman, Surai, & Deans, 2000a; Ruberto & Baratta, 2000); the most effective compounds appear to be those with a phenolic structure, such as eugenol and thymol.

The six essential oils analyzed present a large variety in their composition. Some components were common for different oils, but they were present in large amounts in only a few oils, whereas other components were found just in one oil. For example, eugenol was present in different oils (clove, basil, cinnamon) but its percentage was more than 50% only in clove; conversely safrole and myristicin were peculiar to the nutmeg oil.

All the essential oils tested in the DPPH· assay appeared to be endowed with good antioxidant properties. Herein are reported the findings obtained by using the essential oil concentration able to scavenge almost 50% of the initial [DPPH·] (Table 2); however, the antioxidant efficiency of the various essential oils tested was basically dependent on their concentration (data not shown). When maintained at room temperature, their

Table 1
GC analysis of essential oils kept at room temperature (RT) or exposed to different temperatures for 3 h

Oils	Components (retention time, min)		Percentual content				
			RT	80 °C	100 °C	120 °C	180 °C
Basil	1,8-Cineole	(6.16)	6.71 ± 0.51	6.76 ± 0.05	6.64 ± 0.52	6.87 ± 0.65	6.95 ± 0.62
	Linalool	(19.28)	53.3 ± 3.49	53.2 ± 4.98	53.5 ± 4.81	54.0 ± 5.01	54.2 ± 4.87
	Eugenol	(49.04)	14.2 ± 1.01	14.3 ± 1.28	14.2 ± 1.25	13.9 ± 1.21	13.8 ± 1.25
Cinnamon	β-Caryophyllene	(34.18)	5.03 ± 0.37	4.93 ± 0.39	4.94 ± 0.43	4.86 ± 0.37	4.82 ± 0.41
	Cinnamic Aldehyde	(45.84)	67.9 ± 5.98	68.1 ± 6.03	68.0 ± 6.02	67.5 ± 5.28	67.3 ± 5.84
	Eugenol	(49.09)	6.72 ± 0.54	6.73 ± 0.58	6.75 ± 0.57	6.70 ± 0.54	6.68 ± 0.51
Clove	β-Caryophyllene	(19.33)	7.45 ± 0.55	7.39 ± 0.42	7.38 ± 0.51	6.94 ± 0.52	6.14 ± 0.83
	Eugenol	(46.47)	82.6 ± 4.39	82.9 ± 3.67	83.1 ± 5.31	83.2 ± 6.82	83.6 ± 7.01
	Acetyl-eugenol	(51.77)	8.03 ± 0.35	8.08 ± 0.52	8.13 ± 0.64	8.17 ± 0.56	8.36 ± 0.69
Nutmeg	α-Pinene	(17.29)	22.2 ± 2.01	20.9 ± 1.99	21.2 ± 2.06	22.1 ± 1.86	8.48 ± 0.72*
	β-Pinene	(20.85)	15.1 ± 1.26	14.9 ± 1.28	14.8 ± 1.27	15.2 ± 1.24	7.89 ± 0.59*
	Sabinene	(22.24)	20.2 ± 2.18	19.6 ± 1.62	19.3 ± 1.52	20.1 ± 1.83	2.80 ± 0.19*
	γ-Terpinene	(24.20)	4.10 ± 0.32	3.49 ± 0.28	3.74 ± 0.24	3.57 ± 0.29	5.10 ± 0.41
	Terpinen-4-ol	(43.08)	4.23 ± 0.38	4.65 ± 0.32	4.55 ± 0.29	4.36 ± 0.36	6.79 ± 0.53
	Safrole	(59.64)	1.65 ± 0.12	1.84 ± 0.09	1.83 ± 0.11	1.73 ± 0.13	3.61 ± 0.28*
	Myristicin	(77.73)	9.58 ± 0.82	10.5 ± 0.92	10.7 ± 0.93	9.97 ± 0.82	34.9 ± 2.67*
Oregano	*p*-cymene	(11.47)	11.7 ± 1.01	11.8 ± 1.02	12.1 ± 1.06	12.7 ± 1.04	13.1 ± 1.02
	Thymol	(43.70)	5.03 ± 0.49	5.10 ± 0.47	5.03 ± 0.46	5.03 ± 0.45	5.01 ± 0.38
	Carvacrol	(45.25)	48.9 ± 4.37	48.9 ± 4.61	48.8 ± 4.27	49.0 ± 4.19	49.1 ± 4.23
Thyme	*p*-Cymene	(11.51)	26.1 ± 1.99	26.2 ± 2.11	26.7 ± 2.18	27.6 ± 2.37	29.7 ± 2.87
	Linalool	(21.73)	6.17 ± 0.41	6.07 ± 0.52	6.12 ± 0.59	6.01 ± 0.53	5.68 ± 0.52
	Thymol	(43.97)	45.3 ± 3.62	45.8 ± 3.28	46.3 ± 4.86	45.4 ± 4.68	45.4 ± 4.26

Data are expressed as means ± SD of three experiments and were analyzed by the Student's *t*-test for paired data.
$P < 0.05$ vs. the respective RT.

Table 2
Radical-scavenging activity, determined by the DPPH· test, of essential oils and of eugenol, kept at room temperature (RT) or exposed to different temperatures for 3 h

		% Remaining DPPH·				
		RT	80 °C	100 °C	120 °C	180 °C
Essential oils						
Clove	0.026 (μl/ml)	34.8 ± 2.92	36.6 ± 3.92	40.4 ± 3.58	37.72 ± 3.85	40.3 ± 3.82
Cinnamon	0.065 (μl/ml)	55.3 ± 4.58	53.3 ± 4.56	51.9 ± 4.82	49.24 ± 3.95	44.7 ± 4.16
Nutmeg	0.131 (μl/ml)	51.8 ± 4.78	51.2 ± 4.32	45.1 ± 3.94	44.26 ± 4.14	26.6 ± 1.96*
Basil	0.263 (μl/ml)	42.6 ± 3.94	46.5 ± 4.12	50.0 ± 4.28	55.30 ± 4.82	36.6 ± 3.14
Oregano	0.263 (μl/ml)	51.8 ± 4.71	53.0 ± 4.62	57.50 ± 4.48	57.2 ± 4.93	48.4 ± 4.53
Thyme	1.32 (μl/ml)	42.8 ± 3.71	46.6 ± 4.22	45.15 ± 3.94	46.1 ± 3.99	37.0 ± 3.24
Standard						
Eugenol	28.3 (μM)	43.5 ± 3.65	47.2 ± 3.58	49.8 ± 4.72	49.4 ± 3.56	44.6 ± 4.28

Data are expressed as means ± SD of three experiments and were analyzed by the Student's *t*-test for paired data.
$P < 0.05$ vs. the respective RT.

free radical-scavenger effectiveness in this test was in the following descending order: clove ≫ cinnamon > nutmeg > basil ⩾ oregano ≫ thyme. The different antioxidant activities could be ascribed to the different qualitative/quantitative chemical profiles. However, due to their complex composition, the correlation between antioxidant activity and the components present in the oil is difficult to establish. Many reports on the essential oil activities often refer to concepts, such as synergism, antagonism and additivity, which are rarely experimentally supported, being for the most part purely speculative, but become useful to explain the observed biological activity. One can point out that, in our study, the most effective essential oils appear to be those containing eugenol (clove and cinnamon), in agreement with the concept that the structural feature required for a strong free radical-scavenging activity is a phenolic group containing an electron repelling group in the or-

tho-position to the phenolic group (Dorman et al., 2000a; Dorman, Figueiredo, Barroso, & Deans, 2000b).

Heating (up to 180 °C) of basil, cinnamon, clove, oregano and thyme oils did not influence either their antioxidant activities (being the amount of the remaining DPPH· not significantly affected) or their chemical composition. Conversely, when heated at 180 °C, nutmeg oil showed a significantly higher free radical-scavenging activity, together with a marked loss of α-pinene, β-pinene and sabinene, and an evident increase in safrole and myristicin contents. Thus one could speculate that the observed higher free radical-scavenging capacity of the nutmeg oil might be related to a heating-induced increase in the content of these two components; this hypothesis seems to be confirmed by the dot-blot test on TLC silica layers stained with the free radical DPPH· (Mimica-Dukić, Božin, Soković, Mihajlović, & Matavulj, 2003) (data not shown), although the involvement of other secondary and/or unidentified bioactive compounds cannot be excluded.

Processing methods are generally believed to be responsible for a depletion of naturally occurring antioxidants in raw material of plant origin. Particularly, intense and/or prolonged thermal treatment may be responsible for a significant loss of natural antioxidants, due to the fact that most of the compounds are relatively unstable. However, today it is well known that food processing can have many effects, not all of which result in a loss of quality and health properties (Anese, Manzocco, Vicoli, & Lerici, 1999; Nicoli, Anese, & Parpinel, 1999; Nicoli, Anese, Parpinel, Franceschi, & Lerici, 1997; Yen & Tsai, 1993). In fact, the consequences of food processing and preservation procedures on the overall antioxidant activity of foods are generally the results of different and sometimes opposite events which can take place consecutively or simultaneously. Processing methods may also improve the properties of naturally occurring antioxidants or induce the formation of new compounds having antioxidant properties, so that the overall antioxidant activity of plant raw material can remain unchanged or increased despite the eventual loss of active ingredients. Furthermore, redox reactions occurring between different natural antioxidants and, if antioxidants are mixed with food matrices, between antioxidants and lipid oxidation products, have almost unpredictable consequences on the overall antioxidant properties and food stability (Halliwell, Murcia, Chirico, & Auroma, 1998; Wijewickreme & Kitts, 1998).

In view of a possible employment of spice essential oils in food processing to enhance lipid stability, we have investigated the capability of the essential oils under study to protect α-tocopherol contained in virgin olive oil (a lipid-rich substrate), against thermal oxidative degradation.

The heating at 180 °C for 10 min (an experimental model mimetic of the frying procedure) (Gordon & Kourimska, 1995; Quiles, Ramírez-Tortosa, Gómez, Huertas, & Mataix, 2002) strongly decreases the antioxidant capacity of the oil, as shown by the very low remaining amount of α-tocopherol following the thermic stress. All the essential oils tested appeared able to prevent heat-induced loss of α-tocopherol, so protecting the olive oil against thermal oxidative degradation (Table 3). The findings obtained by addition of 10 μl of essential oil to 200 mg of olive oil show that the antioxidant efficiency of the various essential oils tested was in the following descending order: clove > thyme ⩾ cinnamon > basil ≫ oregano > nutmeg. This activity order, very likely related to the chemical composition of the essential oils under study and to the stability of their components, is different enough from that observed in the DPPH· test, and points out the need to screen plant material by using a bank of in vitro assays before assessing their bioactivity (Dorman et al., 2000b). In particular, the relative efficacies of lipophilic and hydrophilic antioxidants are dependent on the lipid substrate, physical state, antioxidant concentration, oxidation time and temperature, the analytical method used to determine the extent and end point of oxidation; furthermore it is difficult to evaluate natural antioxidants in oils and food emulsion in view of the complex interfacial affinities between air–oil and oil–water interfaces involved (Abdalla & Roozen, 1999). Finally it must be taken into account that water soluble antioxidants could protect lipids better than lipid-soluble antioxidants, due to the so-called polar paradox (Porter, Levasseur, & Henick, 1977).

In conclusion, we have demonstrated that the essential oils of basil, cinnamon, clove, nutmeg, oregano and

Table 3
Protective effect of essential oils (10 μl/200 mg of olive oil) and of eugenol (5 mg/200 mg of olive oil) added to extravirgin olive oil on the decrease, induced by thermal stress (180 °C for 10 min), of α-tocopherol content

	Tocopherol content	
	μg/g	% Basal content
Control	13.9 ± 2.06	6.47
Essential oils		
Clove	132 ± 6.18*	61.5
Thyme	97.4 ± 6.11*	45.3
Cinnamon	9.73 ± 5.24*	43.5
Basil	82.3 ± 4.37*	38.3
Oregano	33.3 ± 2.79*	15.5
Nutmeg	16.6 ± 5.98	7.73
Standards		
Eugenol	134 ± 9.72*	62.1

Data are expressed as means ± SD of three experiments and were analyzed by the Student's *t*-test for unpaired data.

The basal α-tocopherol concentration in the olive oil, maintained under the same experimental conditions but at room temperature, was 215 ± 7.53 μg/g.

$P < 0.05$ vs. control.

thyme, traditionally used for their aromatic properties in the preparation of Mediterranean food, exhibit good properties as free radical-scavengers/antioxidants. This fact can support their use to control lipid oxidation during food processing. Further studies are warranted to better understand the factors influencing their antioxidant activity.

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