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Changes in antioxidant concentration of virgin olive oil during thermal oxidation

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Abstract

The concentration of α-tocopherol, hydroxytyrosol derivatives and tyrosol derivatives was measured in samples of virgin olive oil during thermal oxidation at 60 and 100 °C. Hydroxytyrosol derivatives are the first antioxidants that are lost during thermal oxidation (until a peroxide value of 20–30 meq/kg). Tyrosol derivatives seem to be the most stable compounds, while α-tocopherol has an intermediate rate of loss at low peroxide values and is destroyed at peroxide values from 20 to 50 meq/kg. This means that hydroxytyrosol derivatives are the first compounds to be oxidized, providing therefore oxidative stability to the oil. α-Tocopherol seems to be oxidized after a significant decrease on hydroxytyrosol derivatives content. Tyrosol derivatives are the antioxidants that decrease with the lowest rate, providing the oil with the less antioxidant activity.

Keywords: Virgin olive oil; α-Tocopherol; Phenolic compounds; Heating degradation; Antioxidant activity

1. Introduction

Antioxidants are known to act at different levels in the oxidative sequence involving lipid molecules. The presence of important natural antioxidants in plant food is attracting further interest because of their clear benefits as anticarcinogenic agents and as inhibitors of biologically harmful oxidation reactions in the body. The evidence is accumulating that diets rich in plant antioxidants derived from fruits and vegetables are associated with lower risks of coronary heart disease and cancer (Madhavi, Deshpande, & Salunkhe, 1996).

Extra virgin olive oil is particularly rich in polyphenols. A number of simple and complex phenolic antioxidants have been identified in the polar fraction of virgin olive oil. Both simple and complex phenols were detected with the latter being the most abundant. 3–4-Dihydroxyphenyl ethanol (hydroxytyrosol) derivatives and p-hydroxyphenyl ethanol (tyrosol) derivatives (Angerosa, d'Alessandro, Konstantinou, & Di Giacinto, 1995; Montedoro, Servili, Baldioli, & Miniati, 1992b; Montedoro, Servili, Baldioli, Selvaggini, Miniati, & Macchioni 1993; Tasioula-Margari & Okogeri, 2001b) and lignans (Brenes, Garcia, Garsia, Rios, & Carrido, 1999; Owen, Mier, Giacosa, Hull, Spiegelhalder, & Bartsch, 2000a) predominated among the complex phenols.

Phenolic compounds have been reported as influencing sensory quality (Brenes, Garcia, Garsia, Rios, & Carrido, 1999; Esti, Cinquanta, & La Notte, 1998), as having beneficial biological activity (Visioli, & Galli, 1998; Visioli, Bellomo, & Galli, 1998; Owen et al. 2000b) and oxidative stability of olive oil (Baldioli, Servili, Perretti, & Montedoro, 1996; Chimi, Cillard, Cillard, & Rahmani, 1991; Papadopoulos & Boskou, 1991).

Several studies dealing with the antioxidant activity of phenolic compounds and/or tocopherols of virgin olive oil were performed under accelerated conditions involving elevated temperatures, after the addition of known concentrations of antioxidants to an oil or model substrate. The oxidative stability of virgin olive oils correlates mainly with the concentration of hydrophilic phenols and, in particular with the oleosidic forms of hydroxytyrosol. The predominant phenolic compounds of virgin olive oil have an antioxidant effect which decreases in the order: hydroxytyrosol > oleuropein > tyrosol (Chimi et al., 1991). A sinergistic effect between

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oleuropein, which is a derivative of hydroxytyrosol, and α-tocopherol were reported (Baldioli et al., 1996).

Virgin olive oil contains simple and complex phenolic compounds in amounts ranging between 50 and 1000 mg/kg (Montedoro, Servili, Baldioli, & Miniati, 1992a). The relative proportion of this component depends on several factors such as fruit's variety, location, degree of ripeness (Caponio & Gomes, 2001; Cinquanta, Esti, & La Notte, 1997), extraction procedure (Caponio, Alloggio, & Gomes, 1999; Di Giovacchino, Solinas, & Miccoli, 1994). The type as well as the level of these compounds is therefore an important parameter in evaluating the quality and nutritive value of virgin olive oil. Studies on the changes of simple phenolic compounds, such as tyrosol and hydroxytyrosol during 18 month storage, showed the evolution of simple phenols from complex ones and that tyrosol was stable whereas hydroxytyrosol was distroyed by the end of this period of time (Cinquanta et al., 1997). However, the changes of complex phenolic compounds, at different oxidative stages of virgin olive oil has not been elucidated. The complex phenolic compounds are the major phenolic constituents, higher than 90%, of virgin olive oil phenols (Tasioula-Margari & Okogeri, 2001b).

The aim of this work was to study the concentration of α-tocopherol, hydroxytyrosol derivatives (OHTyr) and tyrosol derivatives (Tyr) in samples of virgin olive oil during thermal oxidation at 60 and 100C, in a wide range of oxidative states. Thus, in-depth knowledge regarding the changes, that phenolic substances undergo, could provide better understanding of how the quality of olive oil is affected over time. The reduction of the above antioxidants, was further related to their antioxidant activity.

2. Materials and methods

2.1. Materials

Olive oil samples from *Lianolia* variety olives, collected in the region of Preveza (Epirus, Greece) were used. The olives were harvested by hand-powered implements (brushes). The oils were extracted by a centrifugation system.

2.2. Reagents and standards

Acetonitrile, methanol, hexane, isopropanol (2-propanol) filtered through a 1.5-μm filter, acetic acid, and water were all of HPLC grade and were purchased from Merck (Darmstadt, Germany). Methanol and hexane for oil extraction were pro-analysis grade and were purchased from Merck (Darmstadt, Germany). The standards dl-α-tocopherol were purchased from Merck (Darmstadt, Germany); tyrosol, was purchased from Sigma-Aldrich (Steinheim, Germany).

2.3. Analytical methods

The peroxide index and free acidity were carried out according to the analytical methods described in (IUPAC, standard methods, 1987). Total phenols were evaluated colorimetrically at 725 nm with the Folin-Ciocalteau reagent (Gutfinger, 1981). OHTyr, (Tyr) and α-tocopherol were evaluated by HPLC following the methodology described by Tasioula-Margari and Okogeri (2001b). α-Tocopherol was also determined according to the method described by Carpenter (1979).

2.4. Thermal oxidation at 60 and 100 °C

Samples A, B, and C with different initial quality indices were used for thermal analysis at 60 and 100 °C. The samples (25 ml) were poured into appropriate glass beakers (5 cm diameter) and then heated to 60 and 100 °C, respectively, in the oven. The oven used was a Universal Oven UM 400, Memmert GmbH + Co. KG, Schwabach, Germany. The temperature of 60 °C used simulates the storage under real conditions, whereas the heating at 100 °C simulates the cooking conditions.

The samples were then removed from the oven (at predetermined time intervals to determine the peroxide value of each sample up until a value of approximately 60 or 70 meq/kg), cooled to room temperature (15 min for 60 °C and 30 min for 100 °C), analyzed for peroxide value and finally stored in the freezer for further analysis, after the addition of nitrogen.

2.5. HPLC apparatus

A Shimadzu model HPLC system (Shimadzu Corporation, Kyoto, Japan) consisting of a solvent delivery module (LC-10AD) with a double plunger reciprocating pump, UV-VIS detector (SPA-10A), column oven (CTO-10A) and 20-μl injection loop was used. The column used was an Apex octadecyl 104 C_{18} (25×0.4 cm ID) with 5 μm packing (Jones Chromatography limited, Colorado, USA). Detection was performed at 280 nm for both phenols and α-tocopherol. The elution solvents used were A (2% acetic acid in water), B (methanol), C (acetonitrile) and D (isopropanol). The samples were eluted according to the following gradient: 95% A/5% B as initial condition; 60% A/10% B/30%C in 8 min; 25% B/75% C in 22 min; 40% C/60% D in 20 min, and this percentage was maintained for 15 min; 25% B/75% C in 2 min and finally 95% A/5% B in 3 min. Flow rate was 1 ml/min. The run was performed at 28 °C. The sample injection volume was 20 μl. Identification of compounds was achieved as previously reported (Tasioula-Margari & Okogeri, 2001a) Concentrations of phenolic compounds and α-tocopherol were calculated from integrated areas of the samples and the corresponding calibration curves of standards (tyrosol and α-

Tocopherol respectively). Data was collected and processed using Class-VP Chromatography Laboratory Automated software (Shimadzu Corporation).

3. Results and discussion

Three different olive oil samples were selected according to their quality characteristics. As shown in Table 1 samples A and B can be characterized as "extra" virgin olive oils (acidity values were lower than 1%) while sample C can be characterized as "fine" virgin olive oil (acidity value 1.5%). Peroxide values (PV) ranged between 6.02 and 14.81 meq/kg. Values of total phenols ranged from 124 to 516 mg/kg expressed as tyrosol. Sample A contains the highest amount of OHTyr and Tyr. Sample B contains a small amount of OHTyr and a lower amount of Tyr than sample A. The initial α-tocopherol contents were similar within samples A and B. Sample C contains no OHTyr and contains the lowest amounts of Tyr and α-tocopherol.

The above samples were oxidized at 60 °C until a peroxide value of about 70 meq/kg was reached, and at 100 °C until a peroxide value of about 100 meq/kg was reached. Figs. 1 and 2 depict the relationship between peroxide values and heating time at 60 and 100 °C respectively. The heating at 60 °C correlates better the oxidation under environmental conditions whereas the heating at 100 °C the oxidation under cooking conditions.

As can be observed, oil oxidation proceeds at a lower rate initially. After that the oxidation rate increases considerably. This first period of time is called the induction period (IP) or induction time (IT). During heating at 60 °C and at PV higher than 70meq/Kg (fig. 1), a steep rise in the slope was observed indicating than the oxidation process predominated, as the rate of peroxide formation was higher than that of decomposition. On the contrary, at 100 °C (Fig. 2) the smooth slope between PV and heating time, indicates that both oxidation and thermal decomposition occur. Based on present data above the induction time (IT) for each olive oil sample has been estimated as the time required for a sample to reach a peroxide value of 70 meq/kg of oil (Table 2).

Table 1
Quality indices of the olive oil samples

Sample	Acid value (%)	PV (meq/kg)	Total phenols (mg/kg)	OHTyr (mg/kg)	Tyr (mg/kg)	α-Toc (mg/kg)
A	0.42	6.02	516	80.96	105.42	175.17
B	0.14	9.33	186	9.59	70.99	170.90
C	1.50	14.81	124	nd	47.59	139.29

Samples B and C, despite differences in their initial peroxide and acid values, show similar deterioration patterns and similar oxidative kinetics, whereas sample A shows the highest stability. The high stability of this sample is due to phenolic compounds content. It is the only sample that contains a significant amount of OHTyr (Table 1). Baldioli et al. (1996) showed that oxidative stability of virgin olive oils correlated mainly with the concentration of hydrophilic phenols and in particular with oleosidic forms of hydroxytyrosol. Their

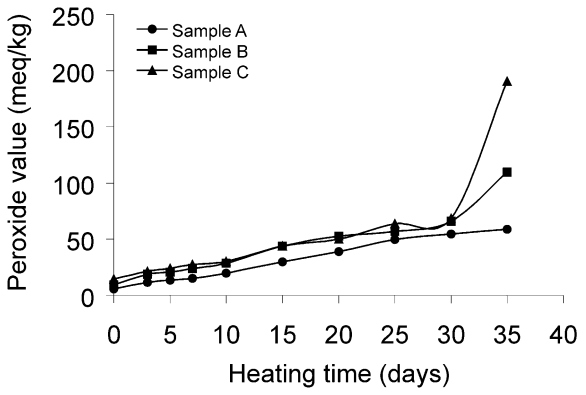

Fig. 1. Relationship between peroxide values and heating time during thermal oxidation at 60 °C.

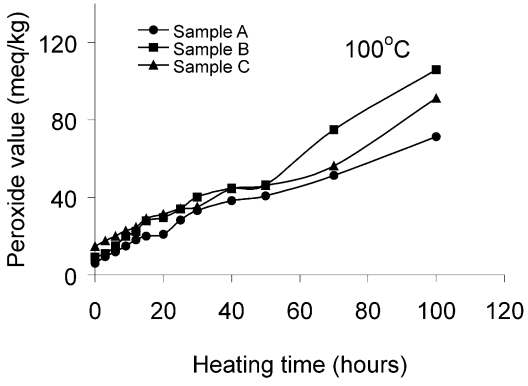

Fig. 2. Relationship between peroxide values and heating time during thermal oxidation at 100 °C.

Table 2
Induction time of the olive oil samples

Temperature (°C)	Sample	Induction time
60		
	A	>35 days
	B	30 days
	C	30 days
100		
	A	100 h
	B	70 h
	C	75 h

results confirmed observations on the low antioxidant activity of tyrosol.

Several samples of each thermally oxidized oil were chosen in order to study the changes in their antioxidant content at different peroxide values. A representative HPLC chromatogram for the determination of phenolic compounds and α-tocopherol is shown in Fig. 3. The main peaks were those with retention time 14.8, 16.8, and 43.7 min, corresponding to OHTyr, Tyr and α-tocopherol respectively.

The peaks of OHTyr, and α-tocopherol are minimized at high peroxide values, while the Tyr appeared as dual peak (Fig. 3b). The peak with a retention time 54.5 min corresponding to triglycerides is increased, owing to the conjugating diene formation during thermal oxidation. A new peak with retention time 25.8 min appeared only at 100°C oxidation.

Tables 3 and 4 contain the changes in antioxidant concentrations of samples at 60 and 100 °C respectively. The samples heated at 60 °C (Table 3) and after 30 days of heating, with a peroxide value ranging between 55 and 68 meq/kg, exhibit high losses in their antioxidant content. Sample A seems to retain a portion of all three antioxidant, while samples B and C seems to have lost their α-tocopherol content, but the last two samples attained a higher peroxide value than the A sample. All three samples retained a high amount of their initial Tyr content.

All of the samples heated at 100 °C (Table 4) and after 100 h of heating, with a peroxide value higher than 70 meq/kg, seem to have lost all of their antioxidant content. An exception to this is the Tyr content, which however is lower, compared to the initial value.

It is also noted that at 60 °C and at the same peroxide value, the heated samples have higher α-tocopherol concentration than at 100 °C. This is probably due to the higher evaporation rate at higher temperatures.

A peroxide value of 20 meq/kg is of interest because above this value virgin olive oil cannot be characterized as "extra". It is thus of equal importance to determine antioxidant concentration until the time that PV reaches this value. Sample A, heated at 60 °C (A2, PV = 19.9

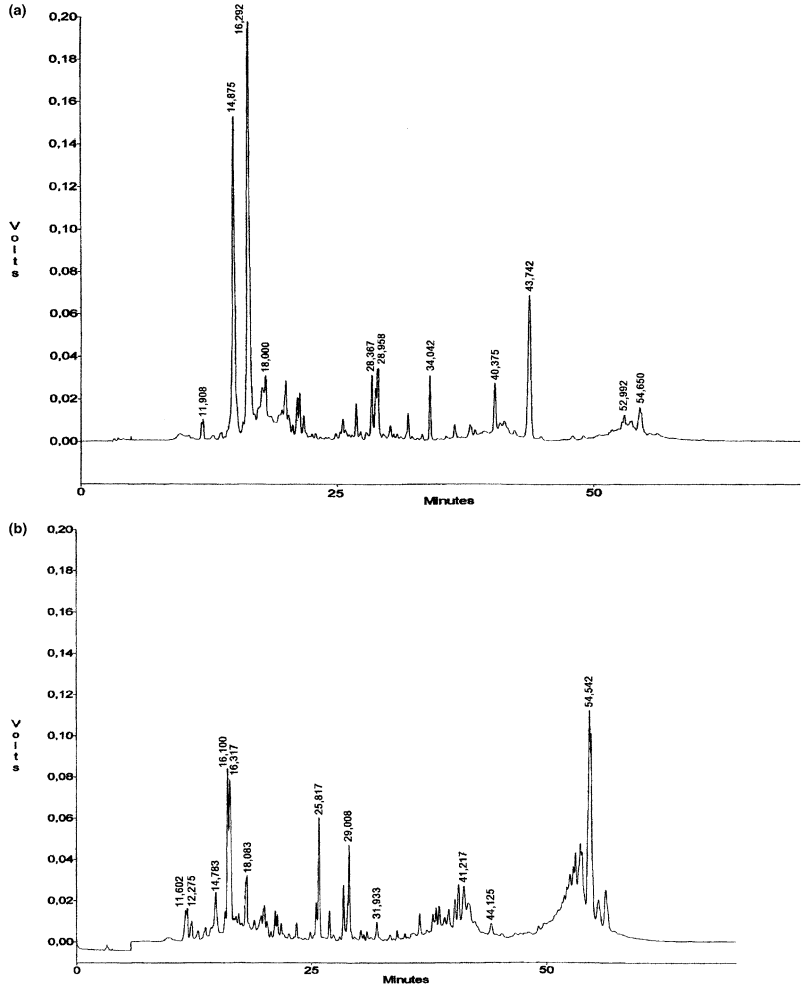

Fig. 3. Representative HPLC chromatogram of phenolic compounds and α-tocopherol separation. (a) initial oil (A0), (b) heated at 100 °C for 100 h (A5). Peak numbers: RT 14.8 min = OHTyr; RT 16.2 min = Tyr; RT 43.7 min = α-Toc.; RT 52.9–54, min = triglycerides.

Table 3
Antioxidant concentrations at various peroxide values during heating at 60 °C

Sample	Heating time (days)	PV (meq/kg)	OHTyr (mg/kg)[a]	Tyr (mg/kg)[a]	α-Toc (mg/kg)
A0	0	6.02	80.96	105.42	175.17
A1	3	11.59	64.72	98.66	168.86
A2	10	19.97	27.41	61.51	154.61
A3	15	30.11	20.83	55.92	117.91
A4	30	54.83	11.69	57.97	56.14
B0	0	9.33	9.59	70.99	170.90
B1	3	18.97	8.09	66.98	170.04
B2	10	28.8	3.67	57.08	156.31
B3	15	43.9	1.98	36.29	98.20
B4	30	65.9	–	35.54	–
C0	0	14.81	–	47.59	139.29
C1	3	21.33	–	39.70	109.14
C2	10	30.21	–	29.35	76.29
C3	15	43.92	–	26.82	10.70
C4	30	68.56	–	13.22	–

[a] Expressed as tyrosol equivalent.

Table 4
Antioxidant concentrations at various peroxide values during heating at 100 °C

Sample	Heating time (hours)	PV (meq/kg)	OHTyr (mg/kg)[a]	Tyr (mg/kg)[a]	α-Toc (mg/kg)
A0	0	6.02	80.96	105.42	175.17
A1	9	14.86	53.46	96.65	175.36
A2	20	21.02	30.06	64.90	126.76
A3	30	33.21	17.66	59.51	60.36
A4	50	40.71	12.61	61.09	9.37
A5	100	71.25	2.75	57.29	–
B0	0	9.33	9.59	70.99	170.90
B1	9	20.09	5.37	67.59	164.84
B2	20	29.70	–	49.53	75.99
B3	30	40.22	–	43.98	–
B4	50	46.37	–	33.11	–
B5	100	105.91	–	32.22	–
C0	0	14.81	–	47.59	139.29
C1	9	22.81	–	45.37	103.84
C2	20	31.25	–	28.77	41.46
C3	30	35.10	–	27.20	–
C4	50	46.04	–	29.05	–
C5	100	91.15	–	12.82	–

[a] Expressed as tyrosol equivalent.

meq/kg) has lost a high amount of its OHTyr (66% reduction), while there is a lower reduction in Tyr and α-tocopherol content. In sample B (B1, PV = 18.9 meq/kg) the low initial OHTyr content is getting lower and no significant changes in Tyr and α-tocopherol occurred. Sample C (C1 PV = 21.3 meq/kg) shows similar behavior with a greater decrease in its α-tocopherol content. This sample contains no OHTyr. The samples, heated at 100 °C show changes in their antioxidant content similar to those of oil heated at 60 °C, until a PV value of 20 meq/Kg (Samples A2, B1, C1, Table 4).

The changes in concentrations of the above antioxidants, during olive oil storage under environmental conditions, showed the same trend. At a mean PV of 20 meq/Kg, OHTyr decreased by 60%, whereas α-tocopherol and Tyr decreased by a lower rate of 38–46% (Okogeri & Tasioula-Margari, in press).

Based on the results obtained in this study, OHTyr is the first antioxidant that is lost during thermal oxidation (until a peroxide value of 20–30 meq/kg). Tyr seems to be the most stable compound, while α-tocopherol has an intermediate rate of loss at low peroxide values and is destroyed at peroxide values from 20 to 50 meq/kg. Chimi et al. (1991) reported that phenolic compounds were degraded as a consequence of their antioxidant activity and their degradation rate was positively correlated to their antioxidant efficacy. This means that OHTyr is the first compound to be oxidized, providing therefore oxidative stability to the oil. α-Tocopherol seems to be oxidized after a significant decrease on OHTyr content. Tyr is the antioxidant that decreases with the lowest rate, providing the oil with the less antioxidant activity.

The above mentioned seem to be in agreement with other studies concerning the behavior of antioxidants in olive oil stability. OHTyr provides the oil with a remarkable stability at 63 °C, but Tyr has practically no antioxidant effect (Papadopoulos & Boskou, 1991). Chimi et al. (1991) reported that phenolic compounds of virgin olive oil have an antioxidant effect which decreases in the order: hydroxytyrosol > oleuropein > tyrosol.

Baldioli et al. (1996) reported that, the oxidative stability of virgin olive oils correlates mainly with the concentration of hydrophilic phenols and, in particular with the oleosidic forms of hydroxytyrosol. The concentration of α-tocopherol, which is quantitatively the most important tocopherol in the oil, shows synergistic activity in mixtures with hydroxytyrosol and its oleosodic forms in purified olive oil.

4. Conclusion

With regard to olive oil stability, OHTyr is the compound required to increase stability of virgin olive oil, as it is effective at peroxide values lower than 20 meq/kg. Tyr and α-tocopherol seem to have a synergistic effect.

With regard to the biological value of virgin olive oil (peroxide value lower than 20 meq/kg) OHTyr is destroyed rapidly and it is possibly absent even in fine virgin olive oil in olive oils after a long storage period. Tyr and α-tocopherol are quite stable up to a peroxide value of 20 meq/kg.

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