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ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · JULY 2005

Impact Factor: 2.91 · DOI: 10.1021/jf0504263 · Source: PubMed

Article

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J. Agric. Food Chem., **2005**, 53 (14), 5766-5771 • DOI: 10.1021/jf0504263

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Relationships between Oxidative Stability, Triacylglycerol Composition, and Antioxidant Content in Olive Oil Matrices

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In olive oils, relationships between oxidative stability, glyceridic composition, and antioxidant content were investigated. Lipid matrices, obtained by purification of olive and high-oleic sunflower oils, were spiked with hydroxytyrosol, α-tocopherol, and mixtures of them and then subjected to oxidation in a Rancimat apparatus at 100 °C. At the same concentration of antioxidants, induction time (IT) decreased as the unsaturation rate of the matrix increased, but only fair correlations were found with fatty acid composition. Oxidative susceptibility (OS_{TAG}) was calculated as a function of the relative oxidation rate of the triacylglycerols, and a linear relationship—IT (h) = $(a + b)OS_{TAG}$—between induction time and this parameter showed a good correlation coefficient ($r > 0.990$, $p < 0.001$). In the case of matrices with a single antioxidant, origin ordinate (a) and slope (b) can be calculated as a function of the antioxidant concentration. In matrices spiked with mixtures of hydroxytyrosol and α-tocopherol, a simple relationship between the coefficients a and b and the concentration of antioxidants cannot be established because additive and subtractive effects occur depending on the relative concentrations of both antioxidants. However, approximate values for these coefficients can be obtained, allowing the estimation of the oil stability. In various olive oils, an acceptable agreement was found between the IT experimentally determined and that calculated from the oil composition. These results confirmed that the Rancimat stability of olive oils mainly depends on triacylglycerol composition and concentrations of *o*-diphenols and α-tocopherol.

KEYWORDS: Hydroxytyrosol; α-tocopherol; antioxidant activity; Rancimat stability; olive oil; triacylglycerol composition

INTRODUCTION

Oxidative stability is an important parameter for evaluating the quality of oils and fats, as it gives a good estimation of their susceptibility to oxidative degradation, the main source of their alteration. The oxidative process depends on exposure to light, temperature, availability of oxygen, glyceridic composition, and the nature and concentration of the antioxidant and prooxidant constituents. However, olive oil stored in-bulk is kept away from light and air, and bottled oil is exposed to light only at retail outlets. Therefore, the main factors affecting the oil shelf life should be the composition of some minor constituents and the glyceridic composition. For the prediction of the olive oil resistance to oxidation under storage conditions, the Rancimat method is one of the most widely used because it is carried out in a commercially available instrument, the procedure is standardized, and results are obtained in a short period of time (*1*). In a previous work (*2*), the antioxidant activity of some minor components of olive oils was investigated using the Rancimat method with a glyceridic matrix obtained by purification of the Picual olive oil variety. At the same millimolar concentration, ortho-diphenolic compounds (hydroxytyrosol, hydroxytyrosyl acetate, aldehydic form of oleuropein aglycon, and luteolin) and mixtures of them resulted in similar antioxidant activities, which were greater than that of α-tocopherol. Tyrosol, squalene, and free fatty acids showed low or negligible effects on oil stability. In the case of mixtures of hydroxytyrosol and α-tocopherol, the stability depended on the concentration ratio between both antioxidants. Other minor constituents of olive oils do not affect significantly the oil stability in darkness (*1*, *2*).

Regarding the glyceridic matrix composition, it is well-known that vegetable oils widely differing in unsaturation rate show different oxidative stabilities (*3*). Studies on fatty acid methyl esters demonstrate that the autoxidation rate of oleic, linoleic, and linolenic methyl esters is 1:40−50:100 (*4*); cis-isomers generally oxidize more readily than trans-isomers, and esters with conjugated double bonds are highly reactive toward oxygen

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10.1021/jf0504263 CCC: $30.25
Published on Web 06/18/2005

(*5*). For mixtures of oleic (O), linoleic (L), and linolenic (Ln) acid methyl esters oxidized at room temperature, the relative rates of oxidation—measured by the formation of hydroperoxides—were 1:10.3:21.6 (*6*). However, the stability of triacylglycerols differs from those of methyl ester mixtures with the same fatty acid composition; thus, olive oil matrices showed higher stability than methyl esters (*7*), whereas the opposite occurred in safflower oil (*8*). Oxidation of synthetic triacylglycerols—at low and high temperatures—showed that triacylglycerol structure plays an important role in the autoxidation rate (*9*−*12*). In addition, the stability of triacylglycerols is affected by the nature and concentration of other triacylglycerols constituting the matrix (*11*, *12*). Consequently, the relationship between stability and fatty acid composition is different for each type of oil; thus, a good correlation between the reciprocal of the stability—determined according to the active oxygen method (AOM)—and linoleic acid contents was found in refined safflower and sunflower oils (*13*), whereas only a fair correlation ($r = 0.85$) between the rate of peroxide formation and percent linolenic acid was reported for matrices obtained from soybean oils (*14*). Olive oils show variable unsaturation degrees because percentages of palmitic, oleic, and linoleic acids are recorded with ranges of 7.0−20.0, 55.0−83.0, and 3.0−21.0%, respectively (*15*). These wide ranges are due to the varietal origin and climatic and agronomical conditions of the orchard where the olive trees are growing. Therefore, the oxidative susceptibility of the glyceridic matrix is an important factor to evaluate the oxidative stability of olive oils.

All of the above antecedents suggest that the stability of olive oils in darkness mainly depends on the concentrations of orthodiphenolic compounds and α-tocopherol and the composition of the glyceridic matrix. In this work, the influence of the lipidic matrix on the Rancimat stability at 100 °C is studied to obtain relationships between the oxidative susceptibility and the triacylglycerol composition. Induction times have been determined in matrices obtained from several olive oils with different triacylglycerol compositions, and matrices spiked with various concentrations of hydroxytyrosol, α-tocopherol, and mixtures of both compounds. Mathematical relationships involving these variables are presented as a first step for the evaluation of the shelf life of olive oils stored in darkness.

MATERIALS AND METHODS

Analytical Materials and Reagents. All solvents and reagents were of analytical grade unless otherwise stated. Neutral alumina, type 507C, grade I, and α-tocopherol were purchased from Fluka AG (Buchs, Switzerland). Silica gel 60 for column chromatography was from Merck KGaA (Darmstadt, Germany).

2-(3′,4′-Dihydroxyphenyl)ethanol (hydroxytyrosol) was synthesized from 3,4-dihydroxyphenylacetic acid (Sigma, Steinheim, Germany) by reduction with $LiAlH_4$ (*16*).

Lipid Matrices. Virgin olive oils (VOO) were obtained from Picual, Hojiblanca, Verdial, and Arbequina olive varieties grown in Spain and from the Picholine variety grown in Morocco and the Chemlali variety grown in Tunisia. Refined high-oleic sunflower oil (HOSO) and two different olive oils of 0.4 and 1.0% of free acidity (as blends of virgin and refined olive oils) were purchased in the market.

Purified VOOs and HOSO were prepared by passing the oils through a chromatographic column packed with aluminum oxide and silica gel as described elsewhere (*2*).

Analytical Determinations. The lipidic matrices were analyzed for free acidity, peroxide index, tocopherols, phenolic compounds, chlorophylls, carotenoids, fatty acid composition, waxes, and squalene as previously described (*2*). Triacylglycerol composition was determined by reverse phase HPLC on an RP-18 column maintained at 20 °C, using propionitrile as mobile phase at a flow rate of 0.6 mL/min, and a refractive index detector (*17*).

Table 1. Fatty Acid Composition of the Glyceridic Matrices Obtained from Virgin Olive Oil (VOO) and High-Oleic Sunflower Oil (HOSO) [Mean Values ($n = 2$)]

	VOO				
fatty acid	Picual	Hojiblanca	Chemlali	Picholine	HOSO
16:0	9.65 ± 0.22	7.95 ± 0.16	12.74 ± 0.32	9.47 ± 0.26	4.46 ± 0.16
16:1 (*n*–7 + *n*–9)	0.84 ± 0.11	0.47 ± 0.02	1.29 ± 0.03	0.68 ± 0.04	0.08 ± 0.01
17:0	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
17:1	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
18:0	3.70 ± 0.03	3.00 ± 0.06	2.61 ± 0.08	2.34 ± 0.09	4.64 ± 0.08
18:1 (*n*–9 + *n*–11)	80.11 ± 0.62	80.03 ± 0.84	67.05 ± 0.45	74.87 ± 0.70	70.94 ± 0.63
18:2 (*n*–9,12)	4.23 ± 0.38	6.80 ± 0.37	14.45 ± 0.36	10.89 ± 0.41	17.90 ± 0.43
18:3 (*n*–9,12,15)	0.48 ± 0.01	0.61 ± 0.10	0.50 ± 0.01	0.88 ± 0.16	0.07 ± 0.01
20:0	0.43 ± 0.01	0.42 ± 0.02	0.67 ± 0.01	0.34 ± 0.04	0.38 ± 0.01
20:1	0.26 ± 0.01	0.40 ± 0.02	0.32 ± 0.01	0.39 ± 0.01	0.24 ± 0.01
22:0	0.12 ± 0.01	0.13 ± 0.01	0.16 ± 0.01	0.02 ± 0.01	1.16 ± 0.02
24:0	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.04 ± 0.01

Oxidative stability was evaluated by an accelerated automated test using the Rancimat apparatus, model CH 9100 (Metrohm Co., Basel, Switzerland). Into Rancimat vessels containing 2.5 g of purified oil were added different amounts of methanolic solution of antioxidants and 0.5 mL of acetone, and then the mixtures were homogenized. The vessels were covered with the heads, placed into the Rancimat apparatus at room temperature, and then heated under an air flow rate of 4 L/h. When the temperature reached 100 °C (∼35 min), the vessels head outlets were connected to the conductivity cells, the air flow rate was increased to 15 L/h, and the measurement time was started. The time taken until there is a sharp increase of conductivity is termed the induction time (IT), and it is expressed in hours. IT was determined by the intersection of the baseline with the tangent to the conductivity curve. All determinations were carried out in duplicate.

For the kinetic study of triacylglycerol oxidation, a mixture of Chemlali VOO (3 g) with trilaurin (0.1 g) was poured into a vessel of the Rancimat apparatus. The oil was subjected to oxidation at 100 °C, bubbling air, until the induction time (26.9 h) was reached. An aliquot (50 mg) of the oxidized oil was purified by passing the aliquot through a silica gel column and eluting with 10 mL of hexane/diethyl ether (87:13), and the solution was evaporated to dryness. The residue was dissolved in 1 mL of acetone and analyzed by HPLC for triacylglycerol determination (*17*).

RESULTS AND DISCUSSION

VOOs obtained from Picual, Hojiblanca, Chemlali, and Picholine olive varieties were chosen to obtain lipidic matrices of different fatty acid compositions. In addition, HOSO was also chosen because it has a fatty acid composition similar to those of olive oils but a different triacylglycerol composition. The purified matrices obtained from these oils contained neither free fatty acids, peroxides, phenols, tocopherols, chlorophylls, nor other minor polar components. In purified VOO matrices, the contents of nonpolar minor compounds were as follows: squalene = 3540−6500 ppm; aliphatic waxes = 90−122 ppm; steroidal waxes = 850−1040 ppm; β-carotene = 0.1 ppm. The purified HOSO contained 123 ppm of squalene. All purified matrices showed a similar content of minor compounds except squalene. Nevertheless, squalene does not affect the Rancimat stability of olive oil matrices (*2*) and, consequently, the induction times depend on only their glyceridic composition. In **Table 1**, it can be seen that percentages of palmitic (%P), oleic (%O), linoleic (%L), and linolenic (%Ln) acids cover the usual range in olive oils (*15*).

To evaluate the influence of the glyceridic matrix on the oxidative stability of olive oils, purified VOOs and HOSO were spiked with hydroxytyrosol and α-tocopherol, in concentration

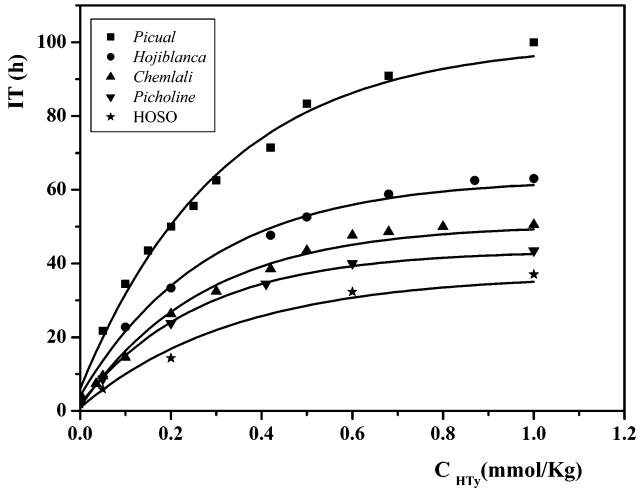

Figure 1. Induction time (IT) of purified virgin olive (Picual, Hojiblanca, Chemlali, and Picholine) and high-oleic sunflower oils (HOSO), spiked with hydroxytyrosol (HTy) in a concentration range of 0.05–1.0 mmol/kg. Stability was determined in a Rancimat apparatus at 100 °C. Mean values ($n = 2$) are shown.

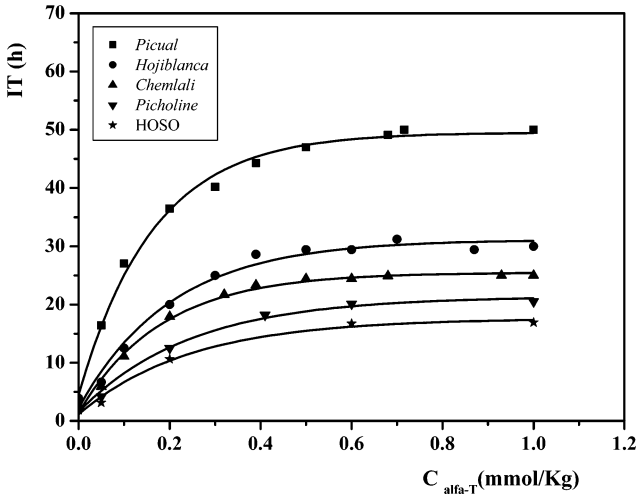

Figure 2. Induction time (IT) of purified virgin olive (Picual, Hojiblanca, Chemlali, and Picholine) and high-oleic sunflower (HOSO) oils, spiked with α-tocopherol (α-T) in the range of concentration between 0.05 and 1.0 mmol/kg. Stability was determined in a Rancimat apparatus at 100 °C. Mean values ($n = 2$) are shown.

(millimoles per kilogram) ranges similar to those of total *o*-diphenols and α-tocopherol found in olive oils (*1*, *18*). Samples were subjected to oxidation in a Rancimat apparatus maintained at 100 °C. Because the Rancimat operates at high temperature, the method provides only an estimation of the oil shelf life because the oxidation mechanism changes from the room temperature to that of the test. Nevertheless, the method provides useful data on the susceptibility to oxidation under the same operating conditions in oils of high stability, such as olive oil matrices containing antioxidants.

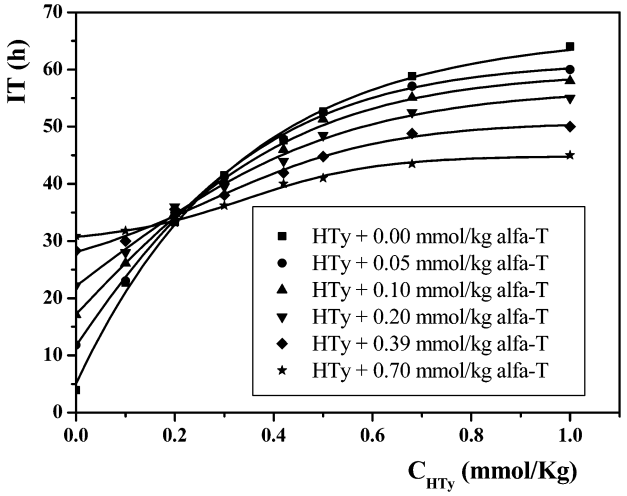

Figure 3. Induction time (IT) of sets of purified Hojiblanca virgin olive oil spiked with α-tocopherol (0.00, 0.05, 0.10, 0.20, 0.39, and 0.71 mmol/kg) and hydroxytyrosol (HTy). Stability was determined in a Rancimat apparatus at 100 °C. Mean values ($n = 2$) are shown.

In matrices containing a single antioxidant, the curves depicting IT versus antioxidant concentration (**Figures 1** and **2**) showed a sigmoid shape, that is, a linear relationship at low concentrations, whereas IT tends toward constant values at high concentrations. In each type of glyceridic matrix, the ITs were higher for those containing hydroxytyrosol than for those with α-tocopherol at similar millimolar concentrations. In matrices without antioxidant added, the differences between IT values are small, and yet upon addition of antioxidants, the ITs spread considerably. In all of the matrices spiked with mixtures of hydroxytyrosol and α-tocopherol, the effect of hydroxytyrosol was influenced by the presence of α-tocopherol; therefore, for concentrations of hydroxytyrosol of <0.2 mmol/kg, the presence of α-tocopherol produced an IT increase lower than the sum of effects due to each antioxidant separately; for hydroxytyrosol concentrations of ~0.2 mmol/kg, α-tocopherol did not produce any effect; and for hydroxytyrosol concentrations >0.2 mmol/kg, the presence of α-tocopherol decreased the IT (see Hojiblanca matrix in **Figure 3** and Picual matrix in **Figure 3** of ref *2*). These results indicated that the previously reported interaction between both antioxidants occurs regardless of the unsaturation rate of the glyceridic matrix (*2*). At the same concentration of antioxidants, the IT value decreased as the unsaturation rate of the matrix increased.

Table 2. Correlation Coefficient (*r*) and Statistical Significance (*p* Level) for the Linear Relationships between Rancimat Induction Time (IT) and Fatty Acid and Triacylglycerol Compositions of the Lipid Matrices[a]

antioxidant concn	1/IT vs L (*13*)		1/IT vs L + 0.07O (*13*)		IT vs O + 10.3L + 21.6Ln (*6*)		IT vs m + 45L + 100Ln (*19*)		IT vs OS_{TAG}[b]	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
0.2 mmol/kg HTy	0.943	0.06	0.843	0.07	0.947	0.06	0.954	0.01	0.996	0.0001
0.5 mmol/kg HTy	0.950	0.01	0.874	0.05	0.914	0.03	0.919	0.03	0.994	0.0006
1.0 mmol/kg HTy	0.933	0.02	0.850	0.07	0.903	0.05	0.906	0.03	0.994	0.0005
0.2 mmol/kg α-T	0.873	0.05	0.810	0.10	0.860	0.10	0.867	0.06	0.988	0.001
0.5 mmol/kg α-T	0.923	0.02	0.833	0.08	0.903	0.05	0.911	0.03	0.987	0.002
1.0 mmol/kg α-T	0.922	0.03	0.842	0.07	0.891	0.08	0.896	0.04	0.986	0.002

[a] L, % linoleic acid; O, % oleic acid; m, % monounsaturated acids; Ln, % linolenic acid; HTy, hydroxytyrosol; α-T, α-tocopherol. [b] Proposed in this work.

Table 3. Relative Oxidation Rate of Triacylglycerols, Oxidative Susceptibility (OS_{TAG}), and Triacylglycerol Composition (Percent) in Matrices Prepared by Purification of Olive Oils (VOO) and High-Oleic Sunflower Oil (HOSO) [Mean Values ($n = 2$)]

HPLC peak	triacylglycerols	relative oxidation rate	VOO				HOSO
			Picual	Hojiblanca	Chemlali	Picholine	
1	LLL	3.0	0.02 ± 0.00	0.05 ± 0.00	0.34 ± 0.03	0.15 ± 0.03	5.24 ± 0.04
2	OLLn + PoLL	2.8	0.06 ± 0.01	0.18 ± 0.03	0.37 ± 0.03	0.38 ± 0.03	0.00 ± 0.00
3	PLLn	2.2	0.01 ± 0.00	0.02 ± 0.00	0.12 ± 0.00	0.09 ± 0.01	0.00 ± 0.00
4	OLL	2.3	0.42 ± 0.02	1.20 ± 0.03	4.0 ± 0.04	2.7 ± 0.03	6.32 ± 0.05
5	OOLn + PoOL	2.3	0.93 ± 0.02	1.23 ± 0.02	1.54 ± 0.03	1.68 ± 0.03	0.31 ± 0.02
6	PLL	2.0	0.12 ± 0.01	0.22 ± 0.02	1.59 ± 0.02	0.57 ± 0.01	2.01 ± 0.03
7	POLn	1.5	0.35 ± 0.03	0.49 ± 0.03	0.88 ± 0.03	0.62 ± 0.03	0.10 ± 0.00
8	OOL	2.2	6.71 ± 0.04	12.81 ± 0.10	14.80 ± 0.10	16.32 ± 0.11	7.72 ± 0.09
9	PoOO	0.7	1.13 ± 0.02	0.74 ± 0.02	1.59 ± 0.03	1.00 ± 0.03	2.43 ± 0.04
10	PLO	0.7	2.59 ± 0.03	3.57 ± 0.04	8.88 ± 0.05	5.65 ± 0.02	2.11 ± 0.03
11	PoOP	0.2	0.55 ± 0.01	0.66 ± 0.02	1.05 ± 0.03	0.48 ± 0.02	0.23 ± 0.01
12	PLP	0.5	*a*	*a*	1.80 ± 0.07	0.81 ± 0.03	*a*
13	OOO	0.7	50.80 ± 1.67	50.24 ± 1.16	29.84 ± 0.81	42.24 ± 1.48	52.20 ± 1.72
14	SOL	0.8	0.79 ± 0.03	1.27 ± 0.04	1.45 ± 0.06	1.42 ± 0.05	1.55 ± 0.06
15	POO	0.2	22.87 ± 0.72	16.92 ± 0.65	19.58 ± 0.52	17.32 ± 0.53	8.12 ± 0.84
16	POP	0.0	2.97 ± 0.04	1.84 ± 0.03	3.93 ± 0.06	2.08 ± 0.05	0.70 ± 0.03
17	SOO	0.2	7.17 ± 0.05	5.68 ± 0.04	3.77 ± 0.04	4.02 ± 0.03	8.55 ± 0.05
18	POS	0.0	1.33 ± 0.03	0.87 ± 0.02	1.03 ± 0.02	0.68 ± 0.02	0.68 ± 0.02
others		0.0	1.22	2.01	3.44	1.79	1.73
	OS_{TAG}		63.7	79.5	87.3	89.9	96.5

[a] Minor peak overlapped by OOO.

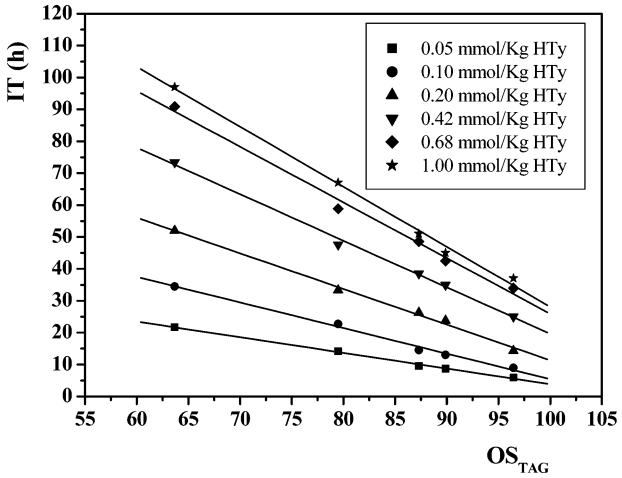

Figure 4. Relationship between the induction time (IT) and oxidative susceptibility (OS_{TAG}) of matrices with different triacylglycerol compositions spiked with different concentrations of hydroxytyrosol (HTy).

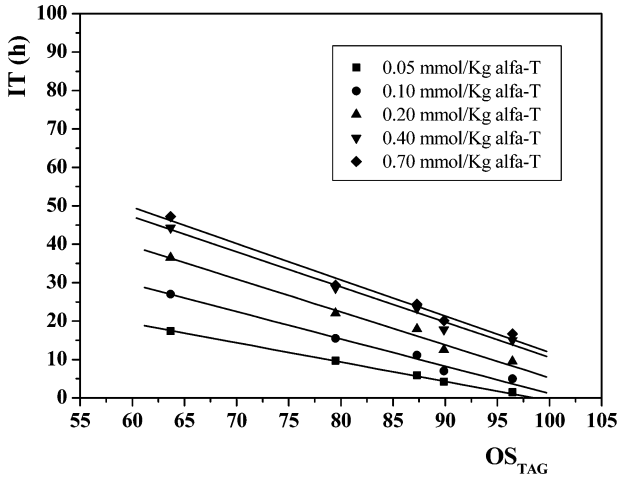

Figure 5. Relationship between the induction time (IT) and oxidative susceptibility (OS_{TAG}) of matrices with different triacylglycerol compositions spiked with different concentrations of α-tocopherol (α-T).

To correlate oxidative stability with the glyceridic composition of vegetable oil matrices, several algorithms including the fatty acid composition have been proposed. Thus, in refined safflower and sunflower oils from plant-breeding trials, relationships between the reciprocal of the induction periods AOM and % L or (% L + 0.07 × % O) were satisfactory (*13*); in purified olive and soybean oils, a relationship between hydroperoxides formed at room temperature and (% O + 10.3 × % L + 21.6 × % Ln) was found (*6*); in virgin olive oils obtained by the "three-phase" centrifugation mode, the Rancimat stability corrected in accordance with the fatty acid composition (% monounsaturated + 45 × % L + 100 × % Ln) showed a fair correlation ($r < 0.9270$) with the concentration of total phenolic compounds (*19*). The application of these mathematical algorithms to matrices spiked with the antioxidants yielded fair correlation coefficients between the induction times measured by Rancimat and the fatty acid compositions (**Table 2**), particularly in matrices containing only α-tocopherol. These results confirm that the fatty acid composition solely does not explain the influence of the matrix because the oxidation rate of an unsaturated acid depends on its position in the triacylglycerol (*9*−*14*, *20*), the nature of other fatty acids constituting the triacylglycerol (*11*), and the proportion of each triacylglycerol in the matrix (*11*, *12*). Therefore, to evaluate the effect of glyceridic matrix on olive oil oxidative stability, the triacylglycerol composition must be taken in account. To calculate the relative oxidation rate of triacylglycerols constituting the olive oils, Chemlali olive oil was spiked with trilaurin (3%) as internal standard and oxidized in a Rancimat apparatus, until the induction time was reached. The oxidized matrix was purified by solid-phase extraction using a silica gel cartridge, and the neutral triacylglycerols were analyzed by high-performance liquid chromatography. From the comparison of the triacylglycerol composition at the induction time with the initial one, relative oxidation rates for each triacylglycerol were calculated (**Table 3**), assuming that trilaurin remains constant.

Table 4. Origin Ordinates and Slopes of the Linear Relationships between Induction Times (IT) at 100 °C and Oxidative Susceptibility (OS_{TAG}) of Lipidic Matrices Spiked with both Variable Amounts of Ortho-diphenolic Compounds and α-Tocopherol [Mean Values ± SD (n = 5)]

o-diphenols (mmol/kg)	α-tocopherol (mmol/kg)	origin ordinate (*a*)	slope (*b*)
0.0	0.05	48 ± 1	–0.49 ± 0.01
	0.10	71 ± 4	–0.69 ± 0.05
	0.20	89 ± 5	–0.83 ± 0.06
	0.40	102 ± 5	–0.92 ± 0.06
	0.70	106 ± 6	–0.94 ± 0.07
0.1	0.05	80 ± 1	–0.69 ± 0.01
	0.10	88 ± 1	–0.78 ± 0.01
	0.20	97 ± 3	–0.80 ± 0.04
	0.40	97 ± 2	–0.83 ± 0.02
	0.70	100 ± 3	–0.85 ± 0.04
0.2	0.05–0.70	117 ± 2	–1.02 ± 0.03
0.3	0.05–0.10	142 ± 3	–1.26 ± 0.04
	0.2–0.70	124 ± 3	–1.08 ± 0.04
0.42	0.05	167 ± 4	–1.49 ± 0.06
	0.10	161 ± 4	–1.44 ± 0.05
	0.20	148 ± 4	–1.29 ± 0.05
	0.40	135 ± 4	–1.16 ± 0.05
	0.70	127 ± 2	–1.09 ± 0.02
0.5	0.05	170 ± 3	–1.47 ± 0.04
	0.10	160 ± 1	–1.37 ± 0.02
	0.20	149 ± 3	–1.26 ± 0.03
	0.40	135 ± 2	–1.13 ± 0.02
	0.70	127 ± 5	–1.07 ± 0.06
0.6	0.05	184 ± 2	–1.61 ± 0.02
	0.10	177 ± 2	–1.55 ± 0.03
	0.20	166 ± 3	–1.45 ± 0.03
	0.40	145 ± 2	–1.23 ± 0.03
	0.70	127 ± 1	–1.06 ± 0.01
0.68	0.05	195 ± 4	–1.75 ± 0.06
	0.10	178 ± 2	–1.55 ± 0.02
	0.20	170 ± 1	–1.48 ± 0.01
	0.40	147 ± 2	–1.24 ± 0.02
	0.70	130 ± 2	–1.08 ± 0.02
≥1.0	0.05	203 ± 3	–1.80 ± 0.04
	0.10	184 ± 1	–1.59 ± 0.01
	0.20	172 ± 1	–1.47 ± 0.01
	0.40	148 ± 3	–1.22 ± 0.04
	0.70	126 ± 1	–1.01 ± 0.01

These results together with the triacylglycerol composition allowed the calculation of the oxidative susceptibility (OS_{TAG}) of the matrix by the formula

$$OS_{TAG} = 3.0(A_1) + 2.8(A_2) + 2.3(A_4 + A_5) + 2.2(A_3 + A_8) + 2.0(A_6) + 1.5(A_7) + 0.8(A_{14}) + 0.7(A_9 + A_{10} + A_{13}) + 0.5(A_{12}) + 0.2(A_{11} + A_{15} + A_{17}) \quad (I)$$

where A_x is the area of each chromatographic peak, expressed as a percentage of the total area, and the numerical coefficients are the relative oxidation rates of each triacylglycerol (**Table 3**).

Application of eq I to the matrices used in this study yielded the OS_{TAG} values shown in **Table 3**, which when applied to matrices spiked with hydroxytyrosol resulted in good linear correlations ($r > 0.994$, $p < 0.0006$) between IT and OS_{TAG} for each concentration of antioxidant (**Figure 4**). In the case of matrices containing only α-tocopherol, the correlation coefficients ($r > 0.986$, $p < 0.002$) were slightly lower (**Figure 5**). In both cases, the correlation with triacylglycerol composition was better than that with fatty acid composition (**Table 2**). In matrices spiked with mixtures of hydroxytyrosol and α-tocopherol, good linear correlations were also obtained ($r > 0.992$, $p < 0.001$).

The relationships between IT and triacylglycerol composition can be summarized by eq II

$$IT\ (h) = (a + b)OS_{TAG} \quad (II)$$

where a and b coefficients depend on the antioxidant concentration. In the case of a single type of antioxidant, the reciprocals of both coefficients ($1/a$ and $1/b$) are related with the reciprocal of the antioxidant concentration by the following equations:

only hydroxytyrosol

$$1/a = 0.00403 + 7.2911 \times 10^{-4} \times (1/C_{HTy}) \quad (r = 0.999,\ p < 0.0001)$$

$$1/b = -0.4622 - 0.0758 \times (1/C_{HTy}) \quad (r = 0.999,\ p < 0.0001)$$

only α-tocopherol

$$1/a = 0.00809 + 5.9681 \times 10^{-4} \times (1/C_{\alpha-T}) \quad (r = 0.999,\ p < 0.0001)$$

$$1/b = -0.9219 - 0.0521 \times (1/C_{\alpha-T}) \quad (r = 0.998,\ p < 0.0001)$$

C_{HTy} and $C_{\alpha-T}$ being concentrations (mmol/kg) of hydroxytyrosol and α-tocopherol, respectively.

In the case of matrices containing a mixture of both antioxidants, a simple mathematical algorithm between coefficients (a and b) and concentrations of hydroxytyrosol and α-tocopherol cannot be established because a positive or negative synergism occurs depending on the relative concentrations of both antioxidants; however, approximate values for a and b coefficients can be obtained by interpolation with the data shown in **Table 4** and then, the theoretical IT can be calculated by applying eq II. The contribution of tyrosol and its derivatives to the oil stability was established as a small increase (~3 h) for concentrations >0.35 mmol/kg (*2*). The application of these

Table 5. Comparison between Induction Times at 100 °C of Olive Oils with the Theoretical Values Calculated Using the Equation IT = (a + b)OS_{TAG} and Data of **Table 4** [Mean Values (n = 2)]

oil	*o*-diphenols (mmol/kg)	α-tocopherol (mmol/kg)	Ty (mmol/kg)	OS_{TAG}	*a*	*b*	induction time (h) theor	induction time (h) exptl
Verdial	0.83 ± 0.02	0.45 ± 0.01	0.95	76.6	147	–1.24	52+(3)[a]	54.0 ± 3.2
Picual	1.38 ± 0.03	0.29 ± 0.01	1.24	59.0	160	–1.35	82+(3)	89.5 ± 5.0
olive oil 1	0.32 ± 0.01	0.45 ± 0.02	0.25	70.1	124	–1.08	48+(1)	46.6 ± 2.1
olive oil 0.4	0.25 ± 0.01	0.39 ± 0.01	0.19	71.0	120	–1.05	45+(1)	43.2 ± 2.4
Arbequina	0.12 ± 0.01	0.35 ± 0.02	0.11	92.7	100	–0.87	19	17.0 ± 2.1

[a] Contribution of tyrosol (Ty) derivatives according to ref *2*.

results to commercial virgin and olive (blend of virgin and refined olive oils) oils resulted in an acceptable agreement between experimental and calculated ITs values (**Table 5**). These results confirm that the Rancimat stability of olive oils mainly depends on triacylglycerol composition and the relationship between concentrations of ortho-diphenolic compounds and α-tocopherol.

ACKNOWLEDGMENT

We are indebted to Rosario González-Cordones and Manuel Rodríguez-Aguilar for technical assistance.

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Received for review February 24, 2005. Revised manuscript received May 17, 2005. Accepted May 23, 2005. We are indebted to CICYT (AGL2000-1481) for financial support.

JF0504263