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Comparison between free radical scavenging capacity and oxidative stability of nut oils

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ABSTRACT

Several works have measured free radical scavenging capacity of nut oils, since they may become a significant source of dietary fat. However, they have not considered kinetic parameters, what was the first aim of this work. Also, it was studied the possible relation between values of free radical scavenging capacity DPPH· and oxidative stability (Rancimat method) in different nut (hazelnut, peanut, pistachio, walnut and almond) oils. The ranking of antioxidant capacity of nut oils, by both assays, was: pistachio > hazelnut > walnut > almond > peanut. A significant correlation was found between DPPH and Rancimat methods assays. Tocopherols appear to be the responsible compounds of this antioxidant capacity being neglictible the contribution of polyphenols. An interference effect of phospholipids, present in methanolic fraction of nut oils, was observed in the determination of polyphenols in nut oils by Folin and *ortho*-diphenols assays.

1. Introduction

The dietary source of fat has proven to be a key aspect in the development of cardiovascular disease and certain kinds of cancer (Aguilera et al., 2003; Kushi, Lenart, & Willett, 1995; Wagner, Tomasch, & Elmafa, 2001). The positive health effects of certain vegetable oils, such as olive oil, are due to their fatty acid composition, rich in monounsaturated fat, that prevent cardiovascular disease by several mechanisms (López-Miranda et al., 2006), and also due to their antioxidants (Ramadan & Moersel, 2006; Tripoli et al., 2005) which prevent the attack of biomolecules by free radicals.

Since nuts contain a high amount of oil (more than 40%), they are a significant potential source of dietary fat; the consumption of nuts in a traditional healthy diet, such as Spanish Mediterranean diet, is approximately 7 g nuts per day (MAPA, 2006). Nut oils are characterized by a high content of mono- and polyunsaturated fat (Maguire, O'Sullivan, Galvin, O'Connor, & O'Brien, 2004). Also, some nut oils exhibit important antioxidant capacity due mainly to their content of tocopherols (Espín, Soler-Rivas, & Wichers, 2000).

Several articles have shown, by DPPH· (2,2-diphenyl-1-picrylhydrazyl) assay, free radical scavenging capacity of heartnut, walnut, peanut, hazelnut and almond oils using different solvents such as ethyl acetate or a mixture hexane/ethyl acetate/methanol (Espín et al., 2000; Li, Rong, Yang, Kramer, & Hernández, 2007; Ramadan & Moersel, 2006). However, antioxidant capacity of the oil from the common pistachio nut has yet to be determined. Moreover, these works have not taken kinetic parameters into account, i.e. the lag time before radicals start to attack susceptible substrates, such as lipids, proteins and DNA. This information together with data concerning their free radical scavenging capacity, provides a more complete parameter of antiradical efficiency (AE) (Sánchez-Moreno, Larrauri, & Saura-Calixto, 1998).

Free radical scavenging capacity is usually measured with the aim of determining possible biological effects of a sample rich in antioxidants since the latter have been related to the prevention of several diseases (Stanner, Hughes, & Buttriss, 2004). In the case of oils, the Rancimat assay is another method commonly used, for determining their oxidative stability, i.e., their half-life. This stability depends mainly on the acylglycerol composition and on the amount and type of minor components in the oil (Mateos, Trujillo,

Abbreviations: AE, antiradical efficiency; AOAC, American Organization of Analytical Chemistry; DMCS, dimethylchlorosilane; DPPH·, 2,2-diphenyl-1-picrylhydrazyl; EC_{50}, concentration to deplete the free radical DPPH· in a 50%; ELSD, evaporative light scattering detector; GC–MS, gas chromatography–mass spectrometry; HMDS, hexamethyldisilazane; HPLC, high performance liquid chromatography; IT, induction time; IUPAC, International Union of Pure and Applied Chemistry; PC, phosphatidylcholine; $t_{EC_{50}}$, time taken by the EC_{50} to reach the steady state; TMS, trimethylsilyl.

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Pérez-Camino, Moreda, & Cert, 2005). The possible relationship between the parameters obtained by these two assays has never before been considered.

The aim of this work was to draw a comparison between the radical scavenging capacity (DPPH· assay) of different nut oils (hazelnut, peanut, pistachio, walnut and almond), considering both activity and kinetics (measured through t_{EC50} parameter), and their oxidative stability as determined by the Rancimat method. Possible correlation between the two assays was also examined. In addition, polyphenol and tocopherol levels were determined in nut oils in order to study their contribution to total antioxidant capacity.

2. Materials and methods

2.1. Chemicals and samples

2,2-Diphenyl-1-picryhydrazyl (DPPH·) was obtained from Panreac, Castellar del Vallés, Barcelona, Spain. Gallic acid was from Sigma–Aldrich Química, S.A., Madrid, Spain. Silica gel column, Folin–Ciocalteau reagent, tocopherol standards, sodium molybdate ($Na_2MoO_4 \times 2H_2O$) were purchased from Merck, Darmstadt, Germany. Hexamethyldisilazane (HMDS), dimethylchlorosilane (DMCS) and pyridine were purchased from Supelco (Bellefonte, PA). All reagents used were of analytical grade.

Samples analyzed included: walnuts (*Juglans regia*) (Iberic walnut Pizarro, Borges S.A., Barcelona, Spain); almonds (*Prunus dulcis*) without shell, hazelnuts (*Corylis avellana)*, peanuts (*Arachis hypogaea*) without shell and pistachios (*Pistachia vera)* (Aperitivos Medina S.L., Mostoles, Madrid, Spain) and a commercial extra virgin olive oil (Carbonell, SOS CUETARA S.A., Madrid, Spain).

2.2. Sample preparation

All samples were milled and ground into a fine powder (particle size $\leqslant$0.5 mm), and then the fat was removed from nuts at room temperature as follows: 0.5 g of milled nut was placed in a test tube and 20 mL of petroleum ether were added. The mixture was thoroughly shaken for 20 min and, then centrifuged at 2500*g* for 10 min. The supernatant was recovered and the solvent evaporated obtaining an oily residue.

A methanolic extraction of nut oils was performed: five millilitres of nut oil were mixed with 5 mL of methanol. The mixture was vigorously stirred for 20 min. and centrifuged at 2500*g* for 10 min and the supernatant was recovered. Another 5 mL of methanol were added and the same process was repeated. Antioxidant capacity was measured by DPPH· method in total nut oils, in the methanolic extracts (“polar fraction”) and in the remaining oil (“non-polar fraction”). Rancimat test was also used in the evaluation of nut oils and in the non-polar fraction-remaining oil after methanolic extraction.

In addition, tocopherols and polyphenols were also determined in nut oils to analyze the possible correlation between these compounds and the nut oils stability.

2.3. Antioxidant capacity

2.3.1. DPPH assay

The method described by Brand-Williams, Cuvelier, and Berset (1995), later modified by Espín et al. (2000) for oils, was used. After adjusting the blank with the corresponding solvent (ethyl acetate for oil and for the non-polar fraction, methanol for the polar fraction), 0.1 mL of the sample was mixed with 3.9 mL of a 60 μM DDPH· solution in the corresponding solvent. The absorbance at 515 nm was measured until the reaction reached the plateau. A calibration curve at that wavelength was made to calculate the remaining DDPH·.

The kinetic parameters described by Sánchez-Moreno et al. (1998) were calculated: EC_{50}, which reflects the depletion of free radical to 50%, was expressed in terms of g dry weight/g DDPH ; $t_{EC_{50}}$, that it is the time taken to reach the steady state at EC_{50}; and the antiradical efficiency, $AE = 1/EC_{50}t_{EC_{50}}$.

2.3.2. Rancimat test

Oxidative stability of nut oils and their non-polar fractions, were evaluated by an accelerated automated test using the Rancimat apparatus, model CH 9100 (Metrohm Co., Basel, Switzerland). Rancimat vessels containing 3 g of oil or non-polar fraction, 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 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 measurement 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 (Frank, Geil, & Freaso, 1982).

2.4. Phenolic compounds

Total polyphenol content in nuts oils was determined in methanolic extract by Folin–Ciocalteau method (Vázquez-Roncero & Janer del Valle, 1973). A calibration curve was done using gallic acid. The results were expressed as mg gallic acid/g oil. *ortho*-Diphenols content in nuts oils was determined in methanolic extact following the method described by Maestro-Durán, Borja, Martín, Fiestas, and Alba (1991). Briefly, 2 mL of methanolic extract was taken and poured into a glass tube. Then, 0.5 mL of a 5% solution of sodium molybdate dihydrate in ethanol/water (1:1, v/v) was added, and the mixture was shacked. After 15 min, the absorbance at λ = 370 nm was measured in a spectrophotometer. Solutions of cathechol, hydroxytyrosol and hydroxytyrosyl acetate were used for build the calibration curve. Results were expressed in millimol *ortho*-diphenols per kg of oil.

2.4.1. Evaluation of phenolic compounds by GC-ion trap-MS spectrometry

The methanolic fraction of nut oils was evaporated to dryness under a stream of nitrogen and the residue was derivatized to its TMS (trimethylsilyl) ethers with 100 μL of a mixture of HMDS:DMCS in pyridine (3:1:9 v/v/v). Aliquots were injected in a trace GC2000 gas chromatograph coupled to a GCQ/Polaris ion trap mass spectrometer (ThermoFinnigan, Austin, TX, USA) according to the process used by Rios, Gil, and Gutierrez-Rosales (2005).

2.5. Determination of fatty acids

For the determination of the fatty acid composition, the methyl esters were prepared by vigorous shaking of a solution of oil in heptane (0.1 g in 3 mL) with 0.2 mL of 2 N methanolic potassium hydroxide. After decantation during 5 min, the upper layer was analyzed by gas-chromatography on a fused silica column (60 m length $\times$ 0.25 mm i.d.) from Merck KGaA (Darmstadt, Germany) coated with SP-2380 phase (0.2 μm thickness) (AOAC, 1990).

2.6. Analysis of tocopherols

Quantification of tocopherols and tocotrienols was carried out following the IUPAC standard method (1992) using high performance liquid chromatography (Hewlett-Packard 1050, CA, United States) on a silica gel column (Merck, Superspher Si60, particle size

4 μm, 25 cm × 4 mm d.i.) with fluorescence detection and external standard solutions of different tocopherols (Merck KGaA, Darmstadt, Germany). Oil samples (100 mg) were dissolved in 10 mL of hexane and directly analyzed. Hexane/2-propanol (99:1, v/v) was used as mobile phase with a flow of 1 mL min^{-1}. The fluorescence detector (Shimadzu RF-535) operated with the excitation wavelength at 290 nm and the emission wavelength at 330 nm. Results were expressed as μg/g oil.

2.7. Determination of phospholipids by HPLC

Methanolic extracts of oils were evaporated and the residue was dissolved in hexane/2-propanol (3:2). This solution was analyzed by HPLC in a Waters 2695 Module (Milford, MA) equipped with an evaporative light scattering detector (Waters 2420 ELSD). Polar and neutral lipids were separated at 30 °C using a Lichrospher 100 Diol 254-4 (5 μm) column (Merck, Darmstadt, Germany) or a normal phase Lichrocart 250-4 (5 μm) column (Merck). In all cases the flow rate was 1 mL min^{-1}, and samples were dissolved in the same solvent as that used to equilibrate the column at the time of injection. Data were processed using Empower software, and the ELSD was regularly calibrated using commercial high-purity standards for each lipid (Salas, Martínez-Force, & Garcés, 2006).

2.8. Statistical analysis

Variance analysis (one-way ANOVA) of DPPH results was carried out by SPSS program version 14.0. Differences were considered statistically significant if probability was greater than 95% (p-value <0.05). Correlation between DPPH of non-polar fraction and total tocopherol were studied by regression line and coefficient of correlation (r).

Extractions were performed by triplicate. Determinations were performed by triplicate and reported on a dry matter. Results are expressed as mean values + SD.

3. Results and discussion

3.1. Antioxidant capacity of nut oils

Table 1 shows DPPH values of nut oils and of the two fractions of oil after extraction with methanol, including kinetic parameters. This method measures the free-radical scavenging capacity of the antioxidants present in the sample.

Walnut oil showed the lowest antioxidant capacity with an EC_{50} of 1514.3 ± 70.2 g oil/g DPPH·, in line with previous published works (Espín et al., 2000). In contrast, hazelnut oil and pistachio oil exhibited a high antioxidant capacity, similar to that of extra virgin olive oil (439.7 ± 8.4 g oil/g DPPH·), the latter also analyzed as an example of oil which is rich in antioxidants. It is remarkable that, despite this important antioxidant capacity, to the authors' knowledge there are no studies on the possible positive health effects derived from bioactive components of pistachio oil.

Kinetic parameters, which had not previously been determined in nut oils, showed that antioxidants from nut oils have slow kinetics with a t_{EC50} between 36 and 78 min compared with the t_{EC50} value of extra virgin olive oil (28.6 min). However, this is an interesting aspect since they could contribute to oil stability over a longer period of time.

The non-polar fraction-remaining oil after extraction with methanol of nut oils exhibited a relatively similar sequence of antioxidant capacity compared to the total oil (pistachio > hazelnut > walnut > almond > peanut). EC_{50} values showed that non-polar fractions have a lower antioxidant capacity than the total oil.

Regarding the methanolic fraction, pistachio oil was again the most antioxidative and exhibited the fastest kinetics. Although the methanolic fraction is not comparable with the values obtained for oil since different solvents are used (measurements in oil are made following dilution with ethyl acetate) and there could be interference affecting antioxidant capacity values (Pérez-Jiménez & Saura-calixto, 2006), t_{EC50} parameters of all polar fractions indicate a quicker ability to scavenge free radicals in this fraction compared with non-polar fractions.

3.2. Antioxidant compounds in nut oils

Tocopherols and polyphenols in nut oils were identified as possibly being the main elements responsible for their free radical scavenging capacity (Table 2).

Table 2
Tocopherols, total polyphenols and *ortho*-diphenols content in nut oils

Sample	Tocopherols[a] (ppm)	Total polyphenols[b]	*ortho*-Diphenols[c]
Walnut oil	249	0.32	0.20
Almond oil	250	0.27	0.70
Hazelnut oil	455	0.08	0.20
Peanut oil	48	0.08	0.00
Pistachio oil	530	0.70	0.37

[a] Sum of α, β, γ and δ tocopherols determined in total oil.
[b] Determined by the Folin method in the methanolic fraction. Expressed as mg gallic acid/g of oil.
[c] Determined by the spectrophotometric method in the methanolic fraction. Expressed as mmol *ortho*-diphenols/kg oil.

Table 1
Antioxidant capacity of nut oils measured by DPPH method

Total oil	Walnut oil	Almond oil	Hazelnut oil	Peanut oil	Pistachio oil
DPPH·					
EC50 (g oil/g DPPH·)	1514.3 ± 70.2[a]	712.2 ± 36[b]	478.5 ± 8.6[b]	1395.9 ± 99.7[a]	377.9 ± 31.8[c]
t_{EC50} (min)	77.8 ± 3[a]	36 ± 2[b]	39.4 ± 2.6[b]	64.9 ± 2[c]	56.9 ± 0.5[d]
AE (×10^6)	8[a]	39[b]	53[c]	11[a]	46[b,c]
Non-polar fraction					
DPPH·					
EC50 (g oil/g DPPH·)	1764.1 ± 125.1[a]	2717.5 ± 68.8[b]	1096.4 ± 52.1[c]	3492.8 ± 53[d]	863.5 ± 41.1[e]
t_{EC50} (min)	20.0 ± 3.0[a]	35.8 ± 0.4[b]	49.2 ± 3.5[c]	57.3 ± 0.6[d]	60.3 ± 2.8[d]
AE (×10^6)	28[a]	10[b]	19[c]	5[b]	19[c]
Methanolic fraction					
DPPH·					
EC50 (g oil/gDPPH·)	688.8 ± 17.5[a]	1109.2 ± 38.8[b]	366.4 ± 60.4[c]	190.2 ± 48.5[d]	8.42 ± 1.5[e]
t_{EC50} (min)	9.8 ± 0.3[a]	20.6[a,b]	24.8 ± 3.7[b]	17.4 ± 6.7[a,b]	20.7 ± 3.2[a]
AE (×10^6)	147[a]	44[a]	110[a]	302[a]	5734[b]

Different letters means statistically differences.

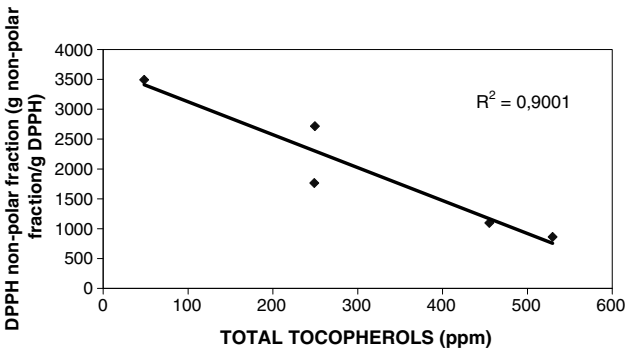

Fig. 1. Correlation between DPPH· of non-polar fraction and total tocopherols (sum of α, β, γ and δ tocopherols) content of nuts oil.

In the case of polyphenols, *ortho*-diphenol content was determined since they have proven to be the most active ones (Gordon, Paiva-Martins, & Almeida, 2001; Papadopoulos & Boskou, 1991).

Hazelnut and pistachio oils contained the highest tocopherol levels consistent with their antioxidant capacity. A significant correlation was found between DPPH· values of non-polar fractions and tocopherol content (Fig. 1, $r^2 = 0.90$, $p = 0.014$), showing that tocopherols exert their antioxidant activity by scavenging free radicals as has previously been described for other samples (Aruoma, 1999). A correlation between DPPH· values and tocopherol content in total oil was not found, probably because compounds extracted with methanol such as phospholipids, contribute to the antioxidant action (Zambiazi, Rui, & Przybylski, 1998). Since it has not been determined that phospholipids may behave as antioxidants in biological systems, DPPH· values determined in non-polar fraction would be more representatives of actual value of the antioxidant capacity of nut oils in order to establish possible health effects of nut oils.

In contrast, no correlation was found between the polyphenol content of the oils measured by spectrophotometric methods and the antioxidant capacity of the methanolic extracts as might have been expected since they exhibit polar characteristics. An attempt was made to determine the presence of phenolic compounds in the methanolic extracts by HPLC-MS (Rios et al., 2005) but results were negative thus contradicting the spectrophotometric values found for polyphenols. Only minute amounts of two phenolic compounds, myricetin and chrysin, have been reported in the polar extract of hazelnut oil (Gordon, Covell, & Kirsch, 2001) but some fatty acid structures were detected. This led to the determination of phospholipids, compounds that may present antioxidant capacity (Ramadan & Moersel, 2006).

Indeed, the methanolic extracts of nut oils contained phospholipids as can be seen in the chromatograms shown in Fig. 2 from almond (0.168 mg PC/mL methanolic fraction of oil), walnut (0.005 mg PC/mL methanolic fraction of oil) and pistachio (0.063 mg PC/mL methanolic fraction of oil).

Since we suspected possible phospholipid interference in the spectrophotometric measurements of polyphenols, analysis of total polyphenols and *ortho*-diphenols was performed with a standard solution of PC in the same way it had previously been carried out in the methanolic extracts of nuts oils. In both assays, phosphatidylcoline provided positive results. This indicates that phospholipids interfere in the measurement of total antioxidants when the Folin method is used, overestimating the phenol value reported in this assay. However, it was not possible to establish the percentual contribution of phospholipids to the spectrophotometric value of polyphenols.

Therefore, the content of polyphenols in nut oils appears to be negligible. For the same reason, *ortho*-diphenol values may be overestimated meaning that these values would not be true.

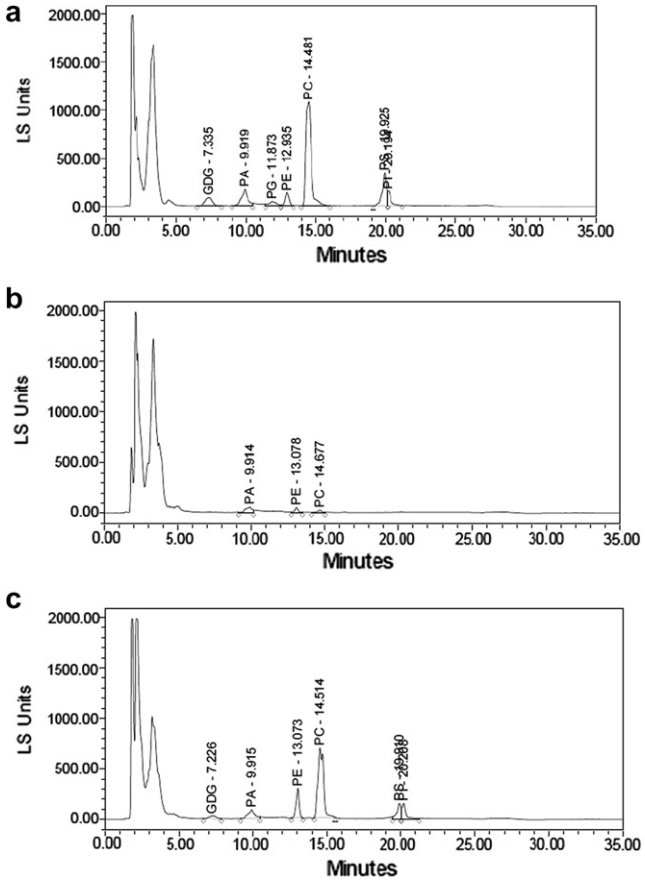

Fig. 2. HPLC chromatograms of polar lipids from almond oil (a), walnut oil (b) and pistachio oil (c) separated on a Lichrospher 100 Diol 254-4 (5 μm) column (Merck). ∗ PC (phosphatidylcholine), PE (phosphatidylethanolamine), PG (phosphatidylglycol), PA (phosphatidic acid), PI (phosphatidylinositol), GDC (monogalactosyldiglyceride), PS (phosphatidylserine).

3.3. Oxidative stability of nut oils

The DPPH· assay measures the ability of the antioxidants present in the sample to scavenge free radicals, an important aspect to consider when measuring the biological activity of these compounds. However, from a technological point of view, the Rancimat technique is usually applied to oils in order to measure their oxidative stability. Table 3 shows the Rancimat assay induction time (IT) value for the nut oils analyzed and for the non-polar fraction of oil remaining after methanolic extraction.

As occurred with the DPPH· assay, the nut oils with the highest stability were hazelnut and pistachio oils, as well as their non-polar fractions.

Rancimat values for nut oils concur with those reported by Savage, Dutta, and McNeil (1999) for walnut oils. Another study on the stability of macadamia oil concluded that stability of this oil

Table 3
Induction time (IT) of nut oils and its non-polar fractions determined by the Rancimat method

	Induction time (h)	
	Total oil	Non-polar fraction
Walnut oil	4.7	4.0
Almond oil	21.8	16.5
Hazelnut oil	52.7	46.1
Peanut oil	14.6	14.5
Pistachio oil	44.4	30.7

Table 4
Fatty acids composition (%) of nut oils

	Walnut oil	Almond oil	Hazelnut oil	Peanut oil	Pistachio oil
Saturated fatty acids (%)	9.39	8.65	7.89	19.81	10.91
16:0	7.04	6.71	5.79	12.06	9.76
18:0	2.27	1.86	2.02	3.62	0.94
20:0	0.08	0.06	0.09	1.37	0.12
22:0	n.d.	0.02	n.d.	1.87	0.07
24:0	n.d.	n.d.	n.d.	0.89	0.02
Monounsaturated fatty acids (%)	26.37	69.65	84.12	42.87	55.03
16:1	0.07	0.60	0.03	0.05	0.78
cis 18:1Δ9	13.45	68.95	81.12	40.78	50.93
trans 18:1Δ9	0.04	0.06	n.d.	0.05	0.04
18:1Δ11	0.97	(a)	2.88	1.41	2.97
20:1	11.85	0.04	0.09	0.58	0.31
22:1	n.d.	n.d.	n.d.	n.d.	n.d.
Polyunsaturated fatty acids (%)	63.24	21.42	7.21	36.86	33.41
18:2	63.19	21.39	7.13	36.79	33.04
18:3	0.05	0.03	0.08	0.07	0.37
Others (%)	1.00	0.29	0.78	0.45	0.65

n.d.: non detected.
(a) Chromatographic peak overlapped by *cis* 18:1Δ9.

was influenced by factors such as the positions of the individual fatty acids within the triacylglycerol molecule and the presence of tocopherols, carotenoids and sterols (Kaijser, Dutta, & Savage, 2000; Neff et al., 1992).

Moreover, the degree of unsaturation of fatty acids also affects the stability of oils, since the more double bonds there are in the fatty acid, the more easily the attack by free radicals take place. For example, the autoxidation rates of oleic, linoleic and linolenic methyl esters are 1:40:100 (Frankel, 1985; Lundberg & Järvi, 1972) which could explain why hazelnut and pistachio oils, which have a higher tocopherol (455.15 and 529.7 ppm, respectively) and oleic acid content (81.1% and 50.9%, respectively), exhibited significant oxidative stability (Tables 2 and 4). On the other hand, the values of IT for non-polar fraction of nut oils were lower than the values for total oil what confirms that phospholipids contribute to the stability of oils.

Finally, a significant correlation was found between DPPH· and Rancimat methods ($r^2 = 0.83$, $p = 0.032$) for total nut oils. This is an important aspect since most results concerning the antioxidant capacity of oils are reported by using the Rancimat method and the possible relationship between this assay and DPPH· had not previously been considered. While a direct comparison is not possible since they focus on different aspects of the antioxidant capacity of oils, this positive correlation indicates that it would be possible to assume that, for a certain batch of samples, the ranking of antioxidant capacity provided by the two assays would be similar.

In summary, a significant correlation existed between free radical antioxidant capacity measured by DPPH· and oxidative stability determined by Rancimat methods in nut oils. Tocopherols appear to be the compounds responsible for this antioxidant capacity. Although a contribution of phospholipids to antioxidant capacity, and therefore to oxidative stability, was found, the biological relevance of it would be uncertain. In the methanolic fraction of the oils, the determination of polyphenols by spectrophotometric methods is interfered by the presence of phospholipids.

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References

Aguilera, C. M., Mesa, M. D., Ramírez-Tortosa, M. C., Nestares, M. T., Ros, E., & Gil, A. (2003). Sunflower oil does not protect against LDL oxidation as virgin olive oil does in patients with peripheral vascular disease. *Clinical Nutrition, 23*, 673–681.
AOAC (1990). *Official methods of analysis*. Washington, DC: AOAC International.
Aruoma, O. I. (1999). Free radicals, antioxidants and international nutrition. *Asia and Pacific Journal of Clinical Nutrition, 8*, 53–63.
Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT, 28*, 25–30.
Espín, J. C., Soler-Rivas, C., & Wichers, H. J. (2000). Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2,2-diphenyl-1-picrylhydrazyl radical. *Journal of Agricultural and Food Chemistry, 48*, 648–656.
Frank, J., Geil, J. V., & Freaso, R. (1982). Automatic determination of oxidation stability of oil and fatty products. *Food Technology, 146*, 71–76.
Frankel, E. N. (1985). Chemistry of free radical and singlet oxidation of lipids. *Progress in Lipid Research, 23*, 197–221.
Gordon, M. H., Covell, C., & Kirsch, N. (2001). Detection of pressed hazelnut oil in admixtures with virgin olive oil by analysis of polar components. *Journal of the American Oil Chemists' Society, 78*, 621–624.
Gordon, M. H., Paiva-Martins, S., & Almeida, M. (2001). Antioxidant activity of hydroxytyrosol acetate compared with that of other olive oil polyphenols. *Journal of Agricultural and Food Chemistry, 49*, 2480–2485.
IUPAC (1992). *Method Num 2432: Determination of tocopherols and tocotrienols in vegetable oils and fats by HPLC Standard methods for the analysis of oils fats and derivatives* (7th ed.). Oxford: Blackwell.
Kaijser, A., Dutta, P., & Savage, G. (2000). Oxidative stability and lipid composition of macadamia nuts grown in New Zealand. *Food Chemistry, 71*, 67–70.
Kushi, L., Lenart, E. B., & Willett, W. C. (1995). Health implications of Mediterranean diets in light of contemporary knowledge 2. Meat, wine, fats and oils. *American Journal of Clinical Nutrition, 61*(Suppl.), 1416s–1427s.
Li, L., Rong, T., Yang, Y., Kramer, J. K. G., & Hernández, M. (2007). Fatty acid profiles, tocopherol contents and antioxidant activities of hearnut (*Juglans ailanthifolia* Var *cordiformis*) and Persian walnut (*Juglans regia* L.). *Journal of Agricultural and Food Chemistry, 55*, 1164–1169.
López-Miranda, J., Badimon, L., Bonanome, A., Lairov, D., Kris-Etherton, P. M., Mata, P., & Pérez-Jiménez, F. (2006). Monounsaturated fat and cardiovascular risk. *Nutrition Reviews, 64*, S2–S12.
Lundberg, W. O., & Järvi, P. (1972). Peroxidation of polyunsaturated fatty compounds. *Progress in Chemistry of Fats Other Lipids, 9*, 377–406.
Maestro-Durán, R., Borja, R., Martín, A., Fiestas, J. A., & Alba, J. (1991). Biodegradation of phenolic compounds present in oil-mill wastewater. *Grasas y Aceites, 42*, 271–276.
Maguire, L. S., O'Sullivan, S. M., Galvin, K., O'Connor, T. P., & O'Brien, N. M. (2004). Fatty acid profile, tocopherol, squalene and phytosterol content of walnuts, almonds, peanuts, hazelnuts and the macadamia nut. *International Journal of Food Science and Nutrition, 55*, 171–178.
MAPA, Ministerio de agricultura, pesca y alimentación (2006). La alimentación en España, 2006. Secretaria general de agricultura y alimentación, Madrid, Spain.
Mateos, R., Trujillo, M., Pérez-Camino, M. C., Moreda, W., & Cert, A. (2005). Relationships between oxidative stability, triacylglycerol composition, and antioxidant content in olive oil matrices. *Journal of Agricultural and Food Chemistry, 53*, 5766–5771.
Neff, W. E., Selke, E., Mounts, T. L., Rinsh, W., Frankel, E., & Zeitoun, M. A. M. (1992). Effect of triacylglycerol composition and structures on oxidative stability of oils from selected soybean germplasm. *Journal of the American Oil Chemists' Society, 69*, 111–118.
Papadopoulos, G., & Boskou, D. (1991). Effect of natural phenols on olive oil. *Journal of the American Oil Chemists' Society, 68*, 669–671.
Pérez-Jiménez, J., & Saura-Calixto, F. (2006). Effect of solvent and certain food constituents on different antioxidant capacity assay. *Food Research International, 39*, 791–800.
Ramadan, M. F., & Moersel, J. T. (2006). Screening of the antiradical action of vegetable oils. *Journal of Food Composition and Analysis, 19*, 838–842.
Rios, J. J., Gil, M. J., & Gutierrez-Rosales, F. (2005). Solid-phase extraction gas chromatography-ion-trap-mass spectrometry qualitative method for evaluation of phenolic compounds in virgin olive oil and structural confirmation of oleuropein and ligstroside aglycons and their oxidation products. *Journal of Chromatography, 1093*, 167–176.
Salas, J. J., Martínez-Force, E., & Garcés, R. (2006). Accumulation of phospholipids and glycolipids in seed kernels of different sunflower mutants (*Helianthus annuus*). *Journal of the American Oil Chemists' Society, 83*, 539–545.
Sánchez-Moreno, C., Larrauri, J. A., & Saura-Calixto, F. (1998). A procedure to measure the antiradical efficiency of polyphenols. *Journal of the Science of Food and Agriculture, 76*, 272–276.
Savage, G. P., Dutta, P. C., & McNeil, D. L. (1999). Fatty acid and tocopherol contents and oxidative stability of walnut oils. *Journal of the American Oil Chemists' Society, 76*, 1059–1063.

Stanner, S., Hughes, J., & Buttriss, J. (2004). A review of the epidemiological evidence for the antioxidant hypothesis. *Public Health Nutrition, 7*, 407–442.

Tripoli, E., Giammanco, M., Tabacchi, G., Di Majo, D., Giammanco, S., & La Guardia, M. (2005). The phenolic compounds of olive oil: Structure, biological activity and beneficial effects on human health. *Nutrition Research Reviews, 18*, 98–112.

Vázquez-Roncero, A., & Janer del Valle, C. (1973). Determination of total polyphenols in olive oils. *Grasas y Aceites, 24*, 350–357.

Wagner, K. H., Tomasch, R., & Elmafa, I. (2001). Impact of diets containing corn oil or olive/sunflower oil mixture on the human plasma and lipoprotein lipid metabolism. *European Journal of Nutrition, 40*, 161–167.

Zambiazi, R., Rui, C., & Przybylski, R. (1998). Effect of endogenous minor components on the oxidative stability of vegetable oils. *Lipid Technology*, 58–62.