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Determination of antioxidant compounds and antioxidant activity in commercial oilseeds for food use

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Abstract

Different commercial edible oilseed samples were analyzed in order to evaluate the compounds involved in antioxidant activity. Fatty acids, triacylglycerols, tocopherols, chlorophylls, β-carotene, squalene, phenolic compounds and CIE $L^*a^*b^*$ coordinates were assayed. Strong chemical variability depending on the oilseed variety was observed. Antioxidant activity was evaluated for the oils, both for their methanol-soluble phase and the fraction insoluble in methanol. DPPH radical scavenging activity expressed in TEAC ranged between 0.45 and 2.30 mmol/l in peanut and maize oils, respectively. Free radical scavenging activity was mainly influenced by tocopherols content ($r = +0.70$) in oils and polyunsaturated fatty acids ($r = +0.61$) in the fraction nonsoluble in methanol. Variability of the correlation between the antioxidant activity and the composition of the oilseeds could be attributed to the differences in the squalene, chlorophylls, carotenoids, and phenols contents of the oils and their mutual interactions.

Keywords: Oilseeds; Composition; Antioxidants; Tocopherols; DPPH radical scavenging activity

1. Introduction

Oilseeds have been part of the diet for a long time and their production has shown a remarkable increase in the last few decades. This development is due to the increasing use of edible fats as vegetable oils and their products of transformation, such as margarine, shortenings, and functional foods. Today's dietary trend is to gradually replace fat from animal origin with those from vegetable, including countries where people traditionally consume fat predominantly from animals. The change is connected with the notion of healthier lifestyle and the need to eat food with a positive influence on health, increasing the use of foods rich in proven beneficial components.

For this reason, improvements in oilseed processing that helps to obtain products with a richer composition, like cold-press extraction, are getting more and more common. Oilseed composition has been studied extensively (Bonaga & Frega, 1997; Caballero, Trugo, & Finglas, 2003; Conte, Frega, Pacetti, & Boselli, 2004), but recently it has been thoroughly investigated taking into account especially the phytochemicals representing the minor components (like tocopherols, squalene, chlorophylls, and phenolic compounds) (Cert, Moreda, & Perez-Camino, 2000; Moreda, Perez-Camino, & Cert, 2001; Shahidi, 2002, 2004a). This interest is connected with the activity of such compounds against cardiovascular diseases (Delplanque et al., 2002; Nielsen, Pedersen, Sandstrom, Marckmann, & Hoy, 2002; Sulli, Sun, Giraud, Moxley, & Driskell, 1998), lipid oxidation, protein cross-linking and DNA mutations (Leckbanda, Frauena, & Friedt, 2002; Moreno & Mitjavila, 2003; Pedersen, Baumstark, Marckmann, Gylling, & Sandström, 2000; Rao, Newmark, & Reddy, 1998; Shahidi, 2004b; Sotiroudis, Kyrtopoulos, Xenakis, & Sotiroudis, 2003), and hemostasis function (Junker et al., 2001). Most of these beneficial effects are due to antioxidant activity: especially when the presence of phenolic compounds and

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tocopherols is involved in the stability of oils (Koski et al., 2002). Unsaturated fatty acids (Delplanque et al., 2002), squalene (Maguire, O'Sullivan, Galvin, O'Connor, & O'Brien, 2004; Newmark, 1997), and carotenoids (Subagio & Morita, 2001) also affect the pro- and/or antioxidative processes in the oils. Nevertheless, the potential help of oilseeds in the everyday diet is difficult to be evaluated due to the insufficiency of the available data (Cabrini et al., 2001; Espín, Soler-Rivas, & Wichers, 2000; Papadopoulos, Triantis, Yannakopoulou, Nikokavoura, & Dimotikali, 2003; Valavanidis et al., 2004) about the antioxidant properties of oilseeds that can be found in normal retailing channels. Moreover, to the best of our knowledge, no data about the antioxidant aspects of flaxseed, grapeseed, and peanut oils have been published. So the aims of the present paper were (a) to characterize the chemical composition of the antioxidant compounds in commercial edible oilseeds, (b) to evaluate their antioxidant activity by DPPH test, and (c) to investigate the correlation between such antioxidant activity and the chemical composition.

2. Materials and methods

2.1. Oil samples

Cold-pressed oilseeds from rape (*Brassica napus* L. var. oleifera), flax (*Linum usitatissimum* L.), sunflower (*Helianthus annus* L.), soya (*Glycine hispida* Moench, *Glycine max (Linne) Mer.*), maize (*Zea mays* L., corn), pumpkin (*Cucurbita pepo* L.), grape (*Vitis vinifera* L.), and peanut (*Arachis hypogaea* L.) were purchased at local grocery stores in Wrocław (Poland) in the years 2004–2005. All oils were produced in Poland, except grapeseed oils which were produced in Tuscany (Italy). Commercial samples of extra-virgin olive oil (*Olea europaea* L.) produced in Tuscany in 2005 were also analyzed.

All samples had been filtered through GD/X 0.45 μm cellulose acetate filters (Whatman, Milan, Italy) before analysis.

2.2. Reagents and standards

Chloroform, methanol, phosphoric acid, *n*-hexane, acetone, and acetonitrile were the analytical grade solvents (Merck, Milan, Italy). Cyclohexane was an analytical UV reagent (Merck). H_3PO_4, KOH, β-carotene, α, δ, and γ-tocopherols (TOCs), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), (±)-6-hydroxy-2,5,7,8-tetramethylcromano-2-carboxylic acid (Trolox), tyrosol, syringic acid, vanillin, vanillic acid, *p*-coumaric acid, ferulic acid, *trans*-cinnamic acid, triacylglycerols (TAGs) (LLL, LLO, LLP, LOO, POL, OOO, POO, POP, SOO), fatty acids methyl esters (FAMEs) (C14:0, C16:0, C16:1$n-7$, C17:0, C18:0, C18:1$n-9$, C18:1$n-7$; C18:2$n-6$, C18:3$n-3$, C20:0, C20:1$n-11$, C22:0, C22:1$n-9$, C22:2$n-6$, C24:0), squalene and squalane were from Fluka and Sigma–Aldrich (Milan, Italy). Oleuropein, luteolin, apigenin, and chlorophyll paste oil were from Extrasynthese (Genay, France). All compounds were analytical grade standards. Oleuropein aglycon was prepared for hydrolysis of the oleuropein with β-glucosidase (Fluka, Milano). HPLC grade water (18 mΩ) was prepared using a Millipore (Bedford, MA, USA) Milli-Q purification system.

2.3. Instrumental analysis

2.3.1. Fatty acids

A transmethylation technique followed by GC-FID determination was used. Fatty acid methyl esters (FAME) were prepared pouring 0.5 g of oil and 250 μl of methanolic 2 M KOH into a 15 ml vial and mixed with a vibration mixer (Falc Instruments, Treviglio, BG, Italy) for 60 s. After 15 min rest, 6 ml of *n*-hexane were poured into the vial and the mixture was shaken in a rotary shaker for 10 min. The layers were allowed to separate and the hexane fraction was injected to GC for analysis. A gas chromatograph HRGC Mega series 2 (Carlo Erba, Milan, Italy) equipped with a FID detector, an AS 800 auto sampler (Carlo-Erba, Milan), a split–splitless injector, and an integrator HP 3396-A (Hewlett Packard, Avondale, PA, USA) was used. The capillary column was a fused silica DB-23 (30 m, 0.25 mm i.d.; 0.25 μm film thickness; J&W Scientific, Fisons, Folsom, CA). The injector and the detector were operated at 240 and 260 °C, respectively. One microlitre of sample was injected in split mode (1:100). The oven was programmed as follows: 100 °C for 1 min, raised to 220 °C (3 °C/min), and from 220 to 250 °C (5 °C/min) and isothermally held for 1 min. Helium was used as carrier gas, and nitrogen as make up at 125 kpa and 80 kpa, respectively. A flame ionization detector (FID) was used with H_2 at 150 kPa, air at 75 kPa, and nitrogen at 90 kPa, respectively. Standard stock solutions were prepared in hexane. The percentage composition of the oils was calculated from GC peak areas without using correction factors.

2.3.2. Squalene

This hydrocarbon was dosed by GC-FID analysis with the FAME. The quantitative analysis was performed using the internal standard method (with squalane) and results were expressed as mg squalene/kg of sample.

2.3.3. Triacylglycerols

For the separation of triacylglycerols (TAG) with UV detection, an HPLC Agilent 1100 (Agilent Technologies, Cernusco sul Naviglio, MI, Italy) fitted with a pump system G1311A, on-line degasser G1322A, auto-sampler G1313A with a 50 μl loop, thermostatted column compartment G1316A (35 °C), and multi diode array detector G1315A with wavelength 207 nm was used. Data acquisition were performed with ChemStation A.08.01 software. Separation was performed with a Kromasil KR100-5 C_{18} column (250 × 4.6 mm, 5 μm, Varian, Leini, TO, Italy) and mobile phase was acetone/acetonitrile (65:35, v/v) with

a flow rate of 0.7 ml/min. Twenty milligrams of oil were diluted to 10 ml with acetone and injected in the HPLC. Standard solution of LLL, LLO, LLP, OOL, POL, OOO, OOP, PPO, and OOS were prepared in acetone. Calibration graphs were constructed with the external standard method by measuring peak area *vs.* concentration. Good linearity for all compounds was achieved and correlation coefficients ranged between 0.9982 and 0.9998. The concentrations of the compounds were calculated in mg/kg and data were expressed in weight percentages. Moreover, the use of equivalent carbon number (ECN) allowed the attribution of compounds of which no analytical standards were found. ECN was calculated according to the equation $ECN = CN - 2n$ where CN is the number of carbon atoms and n is the number of double bonds. In this way LnLL, LnLno, LnLnLn, LLnLn, OLLn, PLLn, OOLn, POLn, LPP, PSL, and SSLn were identified and the quantification was performed using the calibration curves of the TAG standard with the closest chemical structure and ECN number.

2.3.4. α-, γ-, δ-Tocopherols (Vitamin E)

α-, γ-, and δ-Tocopherols stock standard solutions were prepared in acetone, while working solutions were prepared to appropriate dilution with the eluent mobile phase. Ten milligrams of oil were weighed in a 1.8 ml vial, and added with 200 μl of chloroform, and 790 μl of a mixture acetonitrile/methanol (50:50, v/v) and homogenized with a vibration mixer. An HPLC Merck Hitachi 7000 series (Merk, Milan, Italy) connected to a fluorescence detector L-7485 series was used. The operating conditions of fluorescence detector were λex 290 nm and λem 330 nm. Methanol:acetonitrile (50/50, v/v) as eluente mixture was used at a flow rate of 1 ml/min. The column was a Waters ODS2 (250 × 4.6 mm, 0.5μm; Waters, Milan, Italy).

2.3.5. Total chlorophyll and β-carotene

Solutions of 5% of oil in cyclohexane were prepared and absorbance at two different wavelengths were measured (464 nm for β-carotene and 669 nm for total chlorophylls). Chlorophylls and β-carotene stock standard solutions were prepared in cyclohexane, as well as working solutions, that were prepared with proper dilutions. The UV–vis Varian series Cary 50 spectrophotometer was used (see previous).

2.3.6. Phenolic compounds

Samples were prepared by weighing 3 g of oil and adding 5 ml of a methanol–water (80:20 v/v) mixture in a 20 ml screw cap test-tube. The mixture was blended in a vibration mixer apparatus for 1 min, and the emulsion was allowed to separate. The hydrophilic layer, filtered through a GD/X 0.45 μm cellulose acetate septa, was placed in a round flask. Oil extraction was repeated another two times, and the hydrophilic extracts combined and then evaporated under vacuum in a rotary evaporator at 30 °C. The residue was dissolved in 1.5 ml of methanol and injected in the HPLC. An Agilent HPLC system 1100 series (Agilent Technologies, Cernusco sul Naviglio, Milan, Italy) was employed, fitted with a pump module G1311a, an on-line degaser G1322a, an autosampler G1313a with a 20 ml loop and a ThermoSeparation diode array detector SpectroSystem UV 6000lp (ThermoSeparation, San Jose, CA, USA). The solvents used were 0.2 M H_3PO_4 (A) and 80% CH_3CN + 20% 0.2 M H_3PO_4 (B) using linear gradients as follows: $t = 0$ A:B (85:15, v/v), reaching 40:60 (v/v) in 30 min, then 100% B in 10 min, and finally hold at 100% until 50 min. Before each injection the LC system was stabilized for 10 min with the initial A/B ratio (85:15, v/v). The flow rate was 0.8 ml/min. Separation was obtained with a Waters Symmetry C18 column (250 × 4.6 mm, 5 μm; Waters, Milan, Italy). According to the optimal detection wavelength, the phenols analysis was performed at: 280 nm (hydroxytyrosol, tyrosol, syringic acid, vanillic acid, vanillin, oleuropein, and ligstroside), 313 nm (*p*-coumaric acid, ferulic acid, and *trans*-cinnamic acid), and 360 nm (luteolin and apigenin). Chromatograms and spectra were elaborated with a ChromQuest V. 2.51 data system (ThermoQuest, Rodano, Milan, Italy). Stock standard solutions and working solutions were prepared in methanol. Calibration curves were constructed with the external standard method, correlating the area of the peaks with the concentration. The correlation values were comprised between 0.9993 and 0.9999.

2.3.7. CIE $L^*a^*b^*$ coordinates

A UV–vis Varian series Cary 50 Scan spectrophotometer (Varian, Leini, TO, Italy), connected with a PC, was used. Transmittances in a wavelength interval between 380 and 780 nm were measured using D65 illuminant with a 10° observation angle. Data were elaborated with Cary Win UV Color Application V. 2.00 software. The samples were used without any dilution, poured into a 10 mm quartz cuvette.

2.3.8. Radical scavenging activity

A spectrophotometric analysis that used DPPH was performed. This assay is based on the ability of the antioxidant to scavenge the radical cation DPPH. Data were expressed as Trolox equivalent antioxidant capacity (TEAC) using a Trolox calibration curve. The *in vitro* antioxidant activities of oils and both lipophilic (LF) and hydrophilic (HF) fractions were determined. Separation of LF and HF was performed in the following way: 500 μl of seed oil were mixed with 500 μl of methanol in an Eppendorf® microtest tube, shaken in a vibration mixer for 10 s, putted in a rotary shaker for 30 min and then shacked in the vibration mixer for 10 s. After 10 min of centrifugation at 700*g*, the solution was allowed to separate and the methanolic phase with the polar compounds (HF) was separated from the oil (LF). HF was tested using 20 μl of extract added to 3 ml of methanolic DPPH (0.04 mM). LF and a whole oil assay was performed in the same way but using DPPH dissolved in ethyl acetate (Espín et al., 2000; Valavanidis et al., 2004). The spectrophotometric

readings were carried out after a 1-hour period of incubation, in the dark and at room temperature, with a Varian Cary 50 spectrophotometer at 517 nm using a 10 mm quartz cuvette. A Trolox calibration curve in the range 0.02–4.00 mM was prepared, and data were expressed in Trolox equivalent antioxidant capacity (TEAC, mmol/l).

2.4. Statistical analysis

All experiments were replicated three times. Data were evaluated by general linear models with differences between groups being determined using least square means. Analysis of variance (ANOVA) was carried out and the average values were compared with Duncan test. Differences were considered statistically significant at $P < 0.05$. All statistical analyses were performed using GenStat v 7.1 software (VSN International Ltd., Herts, UK).

3. Results and discussion

3.1. Fatty acids and triacylglycerols composition

Tables 1 and 2 show the fatty acids and the triacylglycerols percentage composition. Peanut, rapeseed and olive oil are characterized by the high amount of oleic acid (58.3, 60.7, and 67.2%, respectively). Pumpkin, soybean, maize, sunflower, and grapeseed oils show the typical fatty acid composition with high linoleic acid amount (Bonaga & Frega, 1997; Caballero et al., 2003; Conte et al., 2004; El-Adawy & Taha, 2001; Shahidi, 2002, 2004a). It is interesting to observe that even if two seed oils have a similar fatty acids composition, they can show a quite different TAG profile. For instance, both maize and sunflower oils are rich in linoleic (53.6 and 55.4%), oleic (both 30.2%), and palmitic (12.3 and 6.0%) acids, but maize oil is characterized by LLP, LLO, and LOO (35.8, 22.4, and 14.4%, respectively) and sunflower oil by LLO, LLP, and LLL (25.8, 22.7, and 17.8%). Flaxseed oil shows an unique fatty acid profile, being rich in α-linolenic acid, the essential ω-3 fatty acid. This is confirmed by the high amount of triacylglycerols with an elevated quantity of polyunsaturated fatty acids (PUFA) like LnLnLn, LnLnL, and LnLL (13.1, 12.2, and 15.1%, respectively) in this oil (Jakab, Héberger, & Forgács, 2002) compared to all other oilseeds analyzed. Usually this high amount can be seen as a problem, as it reduces an oil's resistance to oxidation, especially when the oil is used at high temperatures (Chu & Kung, 1998). However, polyunsaturated fatty acids like linoleic and linolenic acid are fundamental in the human diet as they cannot be produced by animal metabolism (Shahidi & Wanasundara, 1998). Linoleic acid is a component of ceramides and it is precursor of arachidonic acid that can produce prostaglandins, thromboxanes, prostacyclin, and leukotrienes (Caballero et al., 2003). Delplanque (2000) suggests that the total daily energy intake should contain 11–16% oleic acid, 4–6% linoleic acid, and 1% α-linoleic acid for best beneficial effects on lipemia and atherothrombotic parameters. Moreover, it should be better to prefer seed oils with low amount of saturated fats like, lauric, myristic and behenic acids because the presence of such compounds can negatively affect human lipid concentrations (Beardsell, Francis, Ridley, & Robards, 2002; Cater & Denke, 2001).

3.2. α-, γ-, δ-Tocopherols

Tocopherols act as antioxidants by trapping the hydroperoxide intermediates and stopping the autoxidation chain reaction. Differences in relative amounts of tocopherols are important: α-TOC affects human nutrition and health aspects, while γ-TOC shows a strong activity in the seed protecting compounds like fatty acids. The amount of tocopherols is very high in maize and soybean

Table 1
Fatty acid composition of vegetable oils (%, w/w, mean ± SD)[a]

Fatty acid			Oil type								
			Flaxseed	Grapeseed	Maize	Peanut	Pumpkin	Rapeseed	Soybean	Sunflower	Olive
M	Myristic	C14:0	*t*	*t*	*t*	*t*	0.1 ± 0.0	*t*	0.1 ± 0.0	0.1 ± 0.0	*t*
P	Palmitic	C16:0	4.9 ± 0.2	6.5 ± 0.4	12.3 ± 0.8	10.0 ± 0.4	10.7 ± 0.6	4.5 ± 0.3	10.2 ± 0.4	6.0 ± 0.2	13.2 ± 0.7
Po	Palmitoleic	C16:1*n* − 7	*t*	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.7 ± 0.0
Ea	Heptadecanoic	C17:0	*t*	*t*	*t*	0.1 ± 0.0	*t*	*t*	0.1 ± 0.0	*t*	*t*
S	Stearic	C18:0	3.7 ± 0.2	3.6 ± 0.2	1.5 ± 0.1	3.3 ± 0.2	5.6 ± 0.3	1.7 ± 0.0	3.7 ± 0.2	3.8 ± 0.2	2.2 ± 0.1
O	Oleic	C18:1*n* − 9	21.3 ± 1.7	17.0 ± 0.9	30.2 ± 2.0	58.3 ± 2.9	34.1 ± 1.9	60.7 ± 2.9	24.6 ± 1.4	30.2 ± 1.9	67.2 ± 3.6
V	Vaccenic	C18:1*n* − 7	0.7 ± 0.1	0.7 ± 0.1	0.5 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	3.2 ± 0.2	1.5 ± 0.1	1.1 ± 0.0	2.6 ± 0.1
L	Linoleic	C18:2*n* − 6	18.1 ± 1.1	70.8 ± 4.6	53.6 ± 3.3	20.9 ± 1.3	47.1 ± 2.6	18.3 ±1.2	50.8 ± 2.8	55.4 ± 4.1	12.5 ± 0.8
Ln	Linolenic	C18:3*n* − 3	50.6 ± 3.2	0.3 ± 0.0	0.7 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	8.1 ± 0.3	7.6 ± 0.4	1.8 ± 0.1	0.7 ± 0.1
A	Arachidic	C20:0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	1.4 ± 0.1	0.4 ± 0.0	0.6 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
Ec	Eicosenoic	C20:1*n* − 11	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	1.0 ± 0.1	0.1 ± 0.1	1.4 ± 0.1	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0
Be	Behenic	C22:0	0.1 ± 0.0	*t*	0.1 ± 0.0	2.5 ± 0.2	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.5 ± 0.0	0.1 ± 0.0
Er	Erucic	C22:1*n* − 9	*t*	*t*	*t*	0.1 ± 0.0	*t*	0.6 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	*t*
Dd	Docosadienoic	C22:2*n* − 6	*t*	*t*	0.1 ± 0.0	0.3 ± 0.0	*t*	*t*	*t*	*t*	*t*
Lg	Lignoceric	C24:0	0.3 ± 0.0	0.6 ± 0.0	0.3 ± 0.0	1.2 ± 0.1	0.6 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.0

t trace < 0.05%.
[a] Values are means of triplicate determinations.

Table 2
Triacylglycerols composition of vegetable oils (%, w/w, mean ± SD)[a]

Triacylglycerols	ECN	Oil type								
		Flaxseed	Grapeseed	Maize	Peanut	Pumpkin	Rape	Soybean	Sunflower	Olive
LnLnLn	36	13.1 ± 2.2	*t*	*t*	*t*	0.2 ± 0.0	0.1 ± 0.0	*t*	0.1 ± 0.0	*t*
LnLnL	38	12.2 ± 1.5	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.6 ± 0.0	1.0 ± 0.0	0.1 ± 0.0	*t*
LnLL	40	15.1 ± 1.4	1.4 ± 0.2	0.7 ± 0.1	1.1 ± 0.1	0.1 ± 0.0	1.5 ± 0.2	6.3 ± 0.7	0.1 ± 0.0	*t*
LnLnO	40	4.8 ± 0.6	*t*	*t*	*t*	*t*	0.6 ± 0.0	*t*	*t*	*t*
LLL[b]	42	2.1 ± 0.3	20.7 ± 2.9	11.8 ± 1.0	3.3 ± 0.2	9.2 ± 1.1	1.9 ± 0.2	12.5 ± 1.5	17.8 ± 1.6	0.2 ± 0.0
LnLO	42	7.7 ± 0.6	*t*	0.4 ± 0.0	0.1 ± 0.0	*t*	7.2 ± 0.6	3.8 ± 0.4	*t*	0.3 ± 0.0
LLPn	42	10.8 ± 1.7	0.4 ± 0.0	*t*	*t*	0.1 ± 0.0	1.1 ± 0.1	4.6 ± 0.4	0.1 ± 0.0	0.1 ± 0.0
LLO[b]	44	2.1 ± 0.3	21.6 ± 2.8	22.4 ± 2.5	6.4 ± 0.5	17.2 ± 1.6	9.5 ± 1.1	14.9 ± 1.2	25.8 ± 2.3	4.4 ± 0.3
LnOO	44	9.0 ± 1.0	*t*	*t*	*t*	*t*	11.7 ± 1.4	*t*	*t*	0.9 ± 0.1
LLP[b]	44	*t*	35.1 ± 3.1	35.8 ± 3.8	6.9 ± 0.8	26.1 ± 2.8	1.5 ± 0.2	34.5 ± 3.1	22.7 ± 2.2	3.4 ± 0.3
LnOP	44	15.2 ± 1.5	*t*	*t*	*t*	*t*	7.1 ± 0.5	*t*	*t*	2.9 ± 0.3
LOO[b]	46	2.3 ± 0.2	6.4 ± 0.7	14.4 ± 1.5	15.9 ± 1.3	11.5 ± 1.1	23.4 ± 2.6	2.2 ± 0.1	8.5 ± 0.7	17.9 ± 1.5
LOP[b]	46	1.7 ± 0.2	5.0 ± 0.4	4.3 ± 0.3	3.2 ± 0.3	5.1 ± 0.4	4.8 ± 0.4	3.7 ± 0.3	4.2 ± 0.3	3.7 ± 0.7
PLP	46	*t*	0.1 ± 0.0	0.8 ± 0.1	1.3 ± 0.1	0.7 ± 0.1	0.5 ± 0.0	0.5 ± 0.0	0.1 ± 0.0	0.6 ± 0.1
OOO[b]	48	0.5 ± 0.1	0.9 ± 0.1	3.7 ± 0.3	31.2 ± 3.4	5.4 ± 0.5	22.9 ± 2.0	0.4 ± 0.0	0.5 ± 0.0	31.5 ± 3.3
POO[b]	48	2.0 ± 0.2	8.1 ± 1.1	5.1 ± 0.5	11.6 ± 0.9	20.1 ± 2.2	4.1 ± 0.3	10.4 ± 1.1	11.4 ± 1.0	21.8 ± 2.0
POP[b]	48	1.4 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	18.4 ± 2.0	4.2 ± 0.9	0.4 ± 0.0	5.1 ± 0.4	8.4 ± 0.9	0.9 ± 0.1
PSL	48	*t*	*t*	0.1 ± 0.0	*t*	*t*	0.2 ± 0.0	*t*	*t*	3.3 ± 0.3
SSLn	48	*t*	*t*	*t*	*t*	*t*	*t*	*t*	*t*	2.7 ± 0.2
SOO[b]	50	*t*	0.1 ± 0.0	0.2 ± 0.0	0.5 ± 0.1	*t*	0.9 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	5.4 ± 0.4

t trace < 0.05%.
[a] Values are means of triplicate determinations.
[b] Compounds identified by comparison with pure standard.

seed oils (1618.4 and 1797.6 mg/kg, respectively) (Table 3). All seed oils show a preponderance of γ-tocopherol (more than 78% of total tocopherols), except grape and sunflower oils where α-tocopherol is the most important (139.2 and 494.2 mg/kg, respectively). Extra-virgin olive oils always show a high level of α-tocopherol, usually 50 times more than γ-tocopherol. Similar values were found in the literature, although variability within each seed oil variety can be observed and explained by genetic factors (Chu & Kung, 1998; Grela & Günter, 1995; Kurilich & Juvik, 1999; Velasco, Fernandez-Martinez, Garcia-Ruiz, & Dominguez, 2002). Usually high amounts of TOCs are associated with PUFA content (Caballero et al., 2003; Shahidi, 2004), but we do not find any significant correlation between the amount of total tocopherols, α-, and γ-tocopherols and the amount of mono- or polyunsaturated fatty acids.

3.3. Total chlorophyll, β-carotene, and squalene

The chlorophylls amount in oilseeds ranges from a minimum of 1.5 mg/kg in peanut oil to a maximum of 30.8 mg/kg in pumpkin oil (Table 3). High amounts can be found also in soybean, rapeseed and grapeseed, but the highest amount is found in olive oil (33.9 mg/kg). β-Carotene shows a similar behaviour, as the amount in pumpkin oil is the highest (5.5 mg/kg), while peanut and sunflower oils are the poorest of all. Presence of chlorophylls is usually associated with high quality oils, especially olive oil, but sometimes it is preferable to avoid high amounts as they can negatively affect the oil stability (Caballero et al., 2003). β-Carotene is the most common and most effective provitamin A and as with other carotenoids it acts as scavenger of singlet oxygen. Both carotenoids and chlorophylls,

Table 3
Lipophile antioxidant compounds of vegetable oils (mg/kg, mean[a] ± SD)

Oil type	α-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total tocopherol	Chlorophylls	β-Carotene	Squalene
Flaxseed	9.5 ± 0.5	575.0 ± 17.0	4.1 ± 0.3	588.5 ± 35.4	3.4 ± 0.1	0.7 ± 0.1	nd
Grapeseed	139.2 ± 6.2	3.2 ± 0.2	0.1 ± 0.0	142.6 ± 6.4	8.4 ± 0.1	0.2 ± 0.1	nd
Maize	49.5 ± 3.7	1522.6 ± 55.1	46.2 ± 3.3	1618.4 ± 62.1	4.9 ± 0.1	0.9 ± 0.1	338.7 ± 11.5
Peanut	73.2 ± 6.7	312.0 ± 19.0	13.4 ± 0.6	398.6 ± 26.3	1.5 ± 0.1	0.1 ± 0.0	1276.0 ± 27.8
Pumpkin	71.4 ± 2.1	423.1 ± 14.8	13.6 ± 0.5	508.1 ± 17.4	30.8 ± 0.2	5.5 ± 0.1	3529.9 ± 61.4
Rapeseed	90.9 ± 3.4	527.6 ± 19.4	6.1 ± 0.3	624.6 ± 23.0	9.4 ± 0.1	1.7 ± 0.1	437.4 ± 18.3
Soybean	92.1 ± 6.7	1432.3 ± 80.6	273.2 ± 12.9	1797.6 ± 100.2	12.0 ± 0.1	0.3 ± 0.1	nd
Sunflower	494.2 ± 15.1	131.0 ± 6.9	9.2 ± 0.3	634.4 ± 22.3	2.3 ± 0.1	0.1 ± 0.0	170.5 ± 6.4
Olive	212.1 ± 4.1	4.8 ± 0.2	nd	216.8 ± 4.3	33.9 ± 0.2	6.9 ± 0.1	5990.0 ± 95.1

nd not detected.
[a] Values are means of triplicate determinations.

although playing a role as an antioxidant, they also act as pro-oxidants because of their instability against light and heat (Subagio & Morita, 2001).

Squalene is a hydrocarbon present in unsaponifiable matter and is present in high amounts in olive oil. This triterpene is a key intermediate in the steroids biosynthetic pathway. It is also regarded as one of the compounds responsible for the beneficial effects against certain type of cancers (Newmark, 1997; Rao et al., 1998; Smith, 2000) although caution must be exercised when evaluating such data (Scolastici, Ong, & Moreno, 2004). Antioxidant activity of squalene is demonstrated against PUFA (Dessi et al., 2002), and it is secondary to that of phenols and tocopherols (Psomiadou & Tsimidou, 1999). Squalene was not detected in flaxseed, grapeseed, and soybean oils (Table 3). In the other oilseeds, the amount of this hydrocarbon varied from 170.5 to 3529.9 mg/kg in sunflower and pumpkin seed oils, respectively; a higher amount was found in olive oil (5990.0 mg/kg)

3.4. Phenolic compounds

Olives and extra virgin olive oil, rich in minor compounds like phenols, are supposed to be effective in cancer prevention (Hashim, Gill, McGlynn, & Rowland, 2005; Owen et al., 2004), and they can reduce the inflammation process in tissues (de la Puerta, Martinez-Dominguez, & Ruiz-Gutierrez, 2000). The molecular structure of phenols is important for their antioxidant activity, as this activity is enhanced by a second hydroxyl or a methoxy group in the *ortho*- or *para*-position. Chromatograms of the phenolic fraction are very different for the eight oilseeds samples. Table 4 reports the only phenolic compounds positively detected. From the UV spectra it can be suggested that some of compounds are derivatives of gallic acid, so a more detailed investigation is necessary. It is interesting to note that most of the phenolic compounds found in olive oil are not present in oilseeds. No evidence of flavonoid presence (like luteolin or apigenin) was found in the analyzed oilseeds. Maize seed oil contained vanillin, *trans*-cinnamic acid and ferulic acid (2.8, 0.9, and 0.5 mg/kg, respectively). Rapeseed oil had a high amount of syringic acid (6.8 mg/kg) and seeds from rape are described as rich in phenolic compounds, mainly sinapic acid (Amarowicz, Raab, & Shahidi, 2003; Naczk, Amarowicz, Sullivan, & Shahidi, 1998).

3.5. DPPH radical scavenging activity of seed oils

Table 5 shows the antioxidant activity of oilseeds expressed in TEAC for oils, and both for their methanol-soluble phase (HF) and the fraction insoluble in methanol (LF). To assess the antioxidant activity, the DPPH assay was chosen because it is one of the widespread methods used and this makes the comparison easier with the data published in the literature. DPPH radical scavenging activity of the oilseeds shows TEAC values ranging between 0.45 and 2.30 mmol/l, with maize and soybean having higher values (2.30 and 1.75 mmol/l, respectively). It can be noted that the LF have always a higher activity than the hydrophilic fraction. Similar results were reported by others, although the data were expressed in a different way (Espín et al., 2000; Valavanidis et al., 2004). Papadopoulos et al. (2003) found that HFs from maize and sunflower oils have an antioxidant activity much lower than HFs of olive oils, while the data in Table 5 show that the differences are not so marked, especially for maize oil. The analysis of the data does not allow finding an univocal correlation between the antioxidant power of oils and their composition. In fact, maize and soya oils show the highest TEAC value, and they also have the highest tocopherol contents, but not of squalene or chlorophyll. Olive oil, in spite of the elevated content of phenolic substances and squalene, does not show a particularly high TEAC value. Maize oil shows the highest antioxidant activity for oil, HF and LF. The ratio LF/HF shows that the LF always

Table 4
Phenolic compounds of vegetable oils (mg/kg, mean ± SD)[a]

Phenols	Oil type								
	Rapeseed	Peanut	Sunflower	Flaxseed	Maize	Soybean	Grapeseed	Pumpkin	Olive
Hydroxytyrosol[b]	nd	nd	nd	nd	nd	nd	nd	nd	3.2 ± 0.7
Tyrosol	nd	nd	nd	nd	nd	nd	nd	nd	7.4 ± 1.1
Syringic acid	6.8 ± 0.5	nd	*t*	nd	nd	nd	nd	nd	nd
Vanillic acid	*t*	nd	*t*	nd	nd	nd	nd	nd	0.4 ± 0.1
Vanillin	nd	nd	nd	2.5 ± 0.2	2.8 ± 0.2	nd	nd	nd	nd
p-Coumaric acid	*t*	nd	nd	nd	nd	nd	nd	1.8 ± 0.2	0.3 ± 0.1
Ferulic acid	1.6 ± 0.2	nd	nd	*t*	0.5 ± 0.1	nd	nd	nd	nd
trans-Cinnamic acid	nd	nd	nd	nd	0.9 ± 0.1	nd	nd	1.0 ± 0.1	nd
Oleuropein	nd	nd	nd	nd	nd	nd	nd	nd	3.8 ± 0.4
Ligstroside[b]	nd	nd	nd	nd	nd	nd	nd	nd	15.6 ± 1.9
Luteolin	nd	nd	nd	nd	nd	nd	nd	nd	4.0 ± 0.5
Apigenin	nd	nd	nd	nd	nd	nd	nd	nd	1.4 ± 0.2

nd not detected.
t trace (<0.05 mg/kg).
[a] Values are means of triplicate determinations.
[b] Tentatively identified according to the uv-vis spectrum and by comparison of retention times with the literature.

Table 5
Radical scavenging activity of vegetable oils (TEAC[A], mmol/l)

Oil type	TEAC[B,C] (mmol/l)						
	OIL (mean ± SD)		HF (mean ± SD)		LF (mean ± SD)		LF/HF
Flaxseed	1.01a	0.07	0.19a	0.03	0.91a	0.04	4.79
Grape	1.43b	0.06	0.26b	0.03	1.23b	0.09	4.73
Maize	2.30c	0.18	0.45c	0.03	1.89c	0.10	4.20
Peanut	0.45d	0.08	0.08d	0.01	0.43d	0.03	5.38
Pumpkin	0.95ae	0.09	0.23b	0.01	0.75e	0.05	3.26
Rapeseed	0.80e	0.08	0.25b	0.03	0.65e	0.06	2.60
Soybean	1.75f	0.10	0.41c	0.02	1.53f	0.07	3.73
Sunflower	1.11a	0.09	0.16a	0.02	1.18b	0.08	7.38
Olive	1.29b	0.11	0.42c	0.03	0.77e	0.04	1.83

[A] TEAC is the millimolar concentration of a Trolox solution having an antioxidant capacity equivalent to that of the dilution of the seed oils.
[B] Values are means of triplicate determinations.
[C] Values within a column for each extract having different letters are significantly different from each other, using Duncan's LSD test ($P < 0.05$).

predominates the HF with olive oil showing a lower value (1.83), while peanut and sunflower seed oils reach 5.38 and 7.38, respectively. This means that the contribution of the LF is very strong, and this is connected with the amount of PUFA as a significant correlation was found ($r = +0.61$) between polyunsaturated fatty acids and LF. Moreover, a negative correlation ($r = -0.63$) existed between monounsaturated fatty acids and LF. More interesting is the correlation between tocopherols and TEAC values of oils and LF: total tocopherols *vs.* TEAC oil value shows a $r = +0.70$, while total tocopherols *vs.* TEAC LF value shows a $r = +0.75$. Of course, variability and anomalies of the correlation between the antioxidant activity and the composition of the oilseeds could be attributed to the differences in the squalene, chlorophylls, carotenoids, and phenols contents of the oils and their mutual interactions.

3.6. CIE $L^*a^*b^*$ coordinates

Refining process highly affects oilseeds chemical composition, so this analysis was performed in order to evaluate the processing grade: the more refined the oils are, the more light coloured they are. Obtained results demonstrate that at least a bleaching step seems have been applied for all the oilseeds, except for pumpkin oil (data not shown). But, even if this oil is the darkest coloured, this does not assure a higher level of antioxidant activity than the other oilseeds. Significant correlations were found in oilseeds only for β-carotene amount and CIE b^* coordinate ($r = +0.63$) and for chlorophylls amount and CIE a^* ($r = +0.53$).

In conclusion, the autoxidation of oils during processing and storage is the major responsible for food quality deterioration, both from in organoleptic and nutritive viewpoints. The analyzed edible commercial oilseeds show interesting radical scavenging activity that can help to neutralize reactive oxygen species. Thus, it could be concluded that cold-pressed oil is a "quite natural" product because oilseeds, when refined, they have their original amount of antioxidant compounds affected to a large extent. Improvement of the nutritional value of oilseeds should be encouraged, as well as investigations into the relevance of the chemical composition of oilseeds and the body's biological function.

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