J Am Oil Chem Soc (2008) 85:141–149
DOI 10.1007/s11746-007-1178-6

ORIGINAL PAPER

Comparison of Two Analytical Methods for Assessing Antioxidant Capacity of Rapeseed and Olive Oils

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Received: 29 June 2007 / Revised: 5 October 2007 / Accepted: 23 November 2007 / Published online: 14 December 2007

Abstract The oxygen radical absorbance capacity (ORAC) and the ferric reducing antioxidant power (FRAP) methods were used for the determination of antioxidant capacities (AC) of rapeseed oils at different steps of technological process and olive oils. The mean ORAC and FRAP results obtained for rapeseed oils (1,106–160 and 552–95.6 μmol TE/100 g) were higher than for olive oils (949–123 and 167–32.1 μmol TE/100 g). Although, FRAP values were lower than ORAC values for all studied oils, there is a linear and significant correlation between these two analytical methods ($r = 0.9665$ and 0.9298, $P < 0.0005$) for rapeseed and olive oils, respectively). Also, total phenolic compounds in rapeseed oils and olives correlated with antioxidant capacities (correlation coefficient ranged between 0.9470 and 0.8049). The refining process of rapeseed oils decreased the total phenolics content and antioxidant capacities by about 80%.

Keywords ORAC and FRAP methods · Rapeseed oils · Olive oils · Refining process

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Introduction

A healthy diet with regard to fats should be based on two approaches: replacement of saturated fats and reduction of dietary cholesterol. Therefore, olive and rapeseed oils are used in cooking, consumption and food production. However, olive oil is typical lipidic source of the Mediterranean diet, whereas rapeseed oil is traditionally consumed in Eastern Europe. It was noted, that the content of monounsaturated fatty acids in both oils is similar. The main difference between olive oil and rapeseed oil comes from the high amounts of omega-3 fatty acids in the latter. This is a special type of polyunsaturated fat, that is believed to provide protection from cardiovascular disease by counteracting thrombosis. Therefore, rapeseed oil is regarded as being the most useful of all cooking fats, because it contains some amount of saturated fat, a lot of monounsaturated fat and a significant fraction of omega-3 fatty acids. Moreover, antioxidant compounds present in these oils including polyphenols, sterols, tocopherols, flavonoids etc. which exhibit antiradical activity. Hence they are important in the prevention and treatment of diseases such as: heart disease, autism, cancer, stroke, diabetes, Alzheimer's dementia, Parkinson's disease, arthritis and muscular degeneration [1].

Recently, different methods based on an electron (ET) and a hydrogen atom transfer (HAT) reaction between a free radical and an oxidant were applied for AC determination of rapeseed and olive oils. Among them, the ET-based methods such as: 2,2′-diphenyl-1-picrylhydrazyl (DPPH) [2, 3], FRAP [1, 4, 5], 2,2′-azinobis (3-ethylbenzothiaziline-6-sulfonate) (ABTS) assays [6] and HAT-based methods: crocin bleaching test [7] and ORAC assay [8–10] were proposed for the evaluation of oils' antioxidant activities. However, only a few reports on FRAP and

ORAC methods for AC determination of rapeseed and olive oils were encountered [1, 4, 5, 8–10]. Only, Hay et al. [10] obtained ORAC values for crude cold pressed rapeseed oil (297 μmol TE/100 g). However, Ninfali et al. [8, 9] used the ORAC test to estimate the antioxidant capacity of extra virgin olive oils (178–700 μmol TE/100 g) and chemical refined olive oil (155 μmol TE/100 g). Moreover, the FRAP method was applied for the determination of AC of rapeseed oils [43–688 μmol Fe(II)/100 g] [4, 5] and olive oils [16–167 μmol Fe(II)/100 g] [1, 5].

Both methods offer some advantages as well as disadvantages which should be eliminated by modification of the analytical procedure. Oxygen radical absorbance capacity assay directly measures the antioxidant activities of chain-breaking antioxidants against peroxyl radicals, which react with a fluorescent probe to form a nonfluorescent product and can be quantitated easily by fluorescence. Therefore, fluorescent markers require detection by fluorimeters, which may not be routinely available in analytical laboratories. Ferric reducing antioxidant power method is based on the reduction of the ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex to the ferrous tripyridyltriazine (Fe^{2+}-TPTZ) at pH = 3.6. This reduction is monitored by measuring the absorption change at 593 nm, thus the FRAP method does not require specialized equipment. However, FRAP measures only the reducing capability based upon the ferric ion, which is not relevant to antioxidant activity mechanistically and physiologically [11]. Moreover, the reaction mechanism for the ORAC method differs from the FRAP method. Therefore, some Authors have discussed the correlation between ORAC and FRAP results for the same sample. A weak but a significant linear correlation was found between serum ORAC and serum FRAP ($r = 0.349$, $P = 0.019$) [12]. Also, ORAC values for the apple extracts correlated with FRAP values ($r^2 = 0.9663$) [13]. However, these different methods do not provide comparable results of antioxidant capacities in the case of common vegetables (r^2 ranged between 0.0055–0.59), except: beet ($r^2 = 0.96$), carrot ($r^2 = 0.78$), purple and white onions ($r^2 = 0.87$ and 0.78, respectively) [14]. Besides, there was no correlation between twenty flavonoids AC determined by these methods ($r^2 = 0.0609$), although a significant correlation between FRAP and ORAC activities for six anthocyanins and three cinnamic acid derivatives was noted ($r^2 = 0.9485$) [15].

However, to the best of our knowledge, there was no reference on correlation evaluation between FRAP and ORAC results for rapeseed and olive oils. The antioxidant capacities of these oils ranged from 16 to 700 μmol/100 g depending on technology and analytical methods used. Therefore, the comparison of different methods will allow a selection of the appropriate analytical procedure of antioxidant capacity determination.

In the presented work ORAC and FRAP methods, after some modifications, were used and compared for the determination of the total antioxidant capacities of rapeseed oils at various stages of technological process and of the olive oils from different regions in Spain and Italy. Therefore, the influence of refining process on the AC of rapeseed oils were examined and compared with the results for the above olive oils. Moreover, total phenolic compounds and tocopherols were analyzed and possible correlations between these parameters and antioxidant capacity were studied and discussed.

Experimental Procedures

Reagents

Reagents were of analytical grade and purchased from POCH (Gliwice, Poland). Fluorescein disodium (FL), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid [Trolox (TE), 97%], 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH, 97%) and 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ, 99%) were supplied by Sigma-Aldrich. Deionized water (DW) was used for the preparation of solutions.

Materials

Ten rapeseed oils from different stages of conventional technological process and nine olive oils were characterized in Table 1. All oils in the original packing [poly(ethylene terephthalate) (PET) or glass bottles] were stored below 10 °C in the dark.

Determination of Fatty Acid Composition

The fatty acid compositions of rapeseed and olive oils were determined according to the official method ISO 5508:1990 [16].

Determination of Tocopherols

Tocopherols content was determined according to the Bunge Europe Research and Development Center—in house method. Oil samples were dissolved in hexane (0.5000 g in 5 mL) and injected (5–20 μL) into a LiChrospher 100 Diol (125 × 4 mm, 5 μm particle size, Agilent Technologies) column and analyzed by an Agilent 1100 HPLC system with an autosampler and fluorescence detector (FLD). The mobile phase was hexane with tetrahydrofuran (96:4 vol/vol%) and

Table 1 List of investigated oils samples

Sample	Type of oil	Type of production	Source
Rapeseed oils			
1	Crude rapeseed oil	Mechanically pressed	Poland
2	Crude rapeseed oil	Mechanically pressed	Poland
3	Degummed rapeseed oil	Mechanically pressed	Poland
4	Neutralized rapeseed oil	Mechanically pressed	Poland
5	Bleached rapeseed oil	Mechanically pressed	Poland
6	Deodorized rapeseed oil	Mechanically pressed	Poland
7	Crude rapeseed oil	Solvent extracted	Poland
8	Crude rapeseed oil	Solvent extracted	Poland
9	Bleached rapeseed oil	Solvent extracted	Poland
10	Deodorized rapeseed oil	Solvent extracted	Poland
Olive oils			
1	Extra virgin olive oil	Mechanically pressed	Spain
2	Extra virgin olive oil	Mechanically pressed	Spain
3	Extra virgin olive oil	Mechanically pressed	Spain
4	Extra virgin olive oil	Mechanically pressed	Italy
5	Olive oil composed of refined olive oil and 40% virgin olive oil	Blending	Spain
6	Olive oil composed of refined olive oil and 20% virgin olive oil	Blending	Spain
7	Olive oil composed of refined olive oil and virgin olive oil	Blending	Spain
8	Olive oil composed of refined olive oil and virgin olive oil	Blending	Italy
9	Refined olive oil	Pressed and chemically refined	Spain

a flow rate of 0.8 ml/min. The excitation and emission wavelengths at 280 and 340 nm were used. The concentrations were calculated from the calibration curves prepared for α-, β-, γ- and δ-tocopherol isomers.

Determination of Total Phenols

Total phenols content (TPC) were determined spectrophotometrically at 725 nm using the Folin-Ciocalteu reagent, according to the procedure described previously by Haiyan et al. [17].

Determination of Antioxidant Capacity

The extracts of oils were obtained in methanol. The test tubes with oils (2.0000–3.0000 g) and methanol (10 mL) were shaken for 60 min at room temperature in the dark. The extracts were then separated from oils in a freezer (−30 °C, 60 min) and transferred quantitatively into a glass bottles. Prior to AC analysis, extracts were stored in refrigerator.

The spectrophotometric FRAP and fluorimetric ORAC methods were used for total AC determination of oils.

Briefly, the FRAP reagent contained 2.5 ml of a 10 mmol/L TPTZ solution in 40 mmol/L HCl, 2.5 ml of 20 mmol/L $FeCl_3$ and 25 ml of 0.1 mol/L acetate buffer (pH 3.6) was freshly prepared and warmed at 37 °C. Then, 0.3 ml of methanolic extracts of oil samples and 2 ml of FRAP reagent were transferred into 10-ml volumetric flask and made up to volume with DW. The blue solutions obtained were kept at room temperature for 6 min and centrifuged at 10,000 rpm for 10 min in a lab centrifuge to remove solids. The absorbance was measured at 593 nm against a reagent blank using a Helios α-UNICAM spectrophotometer in a 1-cm quartz cell.

The reaction mixture for the ORAC assay can be prepared in quartz cuvettes as follows: 1,500 μL of 0.0816 μmol/L FL in 0.075 mol/L phosphate buffer (pH = 7.0), 250 μL of diluted methanolic extract of oil (100 μL into 10 mL volumetric flask) or 250 μL of Trolox standard solutions (0.0031–0.0500 μmol/mL) or blank (phosphate buffer). The mixture was kept 10 min at 37 °C in the dark, and the reaction was initiated by addition of 250 μL of 153 mmol/L AAPH. The fluorescence decay was measured at 37 °C every 1 min at 525 nm emission and 485 nm excitation, using a Hitachi F-4500 Fluorescence Spectrophotometer. A calibration curve was generated using the net area under the curve of FL decay in the presence of five standard concentrations of Trolox (AUC_{TE}) minus AUC_{blank} for blank. $ORAC_{oil}$ values were obtained from the following linear relationship: f(concentration of TE) = ($AUC_{TE} - AUC_{blank}$) after subtracting the AUC_{blank}. Data were expressed as micromoles of TE equivalents per 100 g of oil samples.

Calibration curves were prepared using working solutions of Trolox between 1.00×10^{-3}– 2.00×10^{-2} and 3.91×10^{-4}– 6.25×10^{-3} μmol/mL for FRAP and ORAC methods, respectively. The least-squares method was applied to calculate the lines $y = 38.84 \pm 0.734x + 0.0176 \pm 0.0083$ and $y = 6622.3 \pm 99.13x - 0.9044 \pm 0.3417$ with a correlation coefficient of 0.9996 and 0.9999 for FRAP and ORAC methods, respectively. The relative standard deviations (RSD, $n = 5$) of the slopes were 2.76% for FRAP and 2.16% for ORAC method. The obtained values of RSD indicating reasonable repeatability of both methods.

The calculated detection limit (DL = 4.84×10^{-4} μmol/mL) and quantification limit (QL = 1.61×10^{-3} μmol/mL) for FRAP and DL = 8.22×10^{-5} and QL = 2.74×10^{-4} μmol/mL for ORAC methods confirm linearity concentrations range for AC determinations of the studied oil samples.

Statistical Analysis

The antioxidant capacities of the studied oils were determined (five portions of each oil extracted with MeOH, analyzed within 1 day) by the FRAP and ORAC methods. The results of AC and TPC obtained were presented as: mean (c) ± standard deviation (SD). The Pearson correlation test was used to determine the correlations between variables: FRAP, ORAC results, total phenols and tocopherols content for different rapeseed oils and olive oils. Mean differences were considered significant at the $P < 0.05$ level.

Principal component analysis (PCA) was performed for the results of AC, TPC, total tocopherols content (TTC) and fatty acid composition of rapeseed and olive oils using the Statistica (Windows software package).

Results and Discussion

Composition Analysis of Rapeseed and Olive Oils

Fatty acid composition of the rapeseed and olive oils studied are listed in Tables 2 and 3. Fatty acid profiles are within the official ranges for these oils specified in the Codex Alimentarius [18, 19], thus the results obtained do not require any additional comments. However, it can be noted that the fraction of the saturated fatty acids (SAFA) in rapeseed oils (7.2–7.4%) was comparable, but two times lower than in all olive oils (14.1–17.4%). In addition, the monounsaturated-to-polyunsaturated fatty acid ratio (MUFA/PUFA) for all rapeseed oils was fairly constant (2.1–2.3) and significantly lower in comparison with the MUFA/PUFA ratio for the olive oils discussed (7.1–15.1). The ratio varies widely according to olive variety (Table 3). This is one of the main reasons for the better stability of olive oils with respect to rapeseed oils.

Moreover, composition of individual tocopherols in rapeseed and olive oils were in good agreement with the values proposed by the Codex Alimentary Standard [18, 19]. As can be seen, olives contained only α-tocopherol, whereas γ-and δ-tocopherols were determined in the rapeseed oils analyzed (Tables 2, 3). Therefore, the total contents of tocopherols (TTC) in rapeseed oils (555–690 mg/kg) were a significantly higher in comparison with olive oils (80–190 mg/kg). It can be noted, that refining processes of rapeseed oil decrease γ-tocopherol concentration (about 10%). For comparison, in Kania's work [2], the highest losses of α-tocopherols occurred in degumming and neutralization steps of soybean oil refining (about 25%), whereas the β isomer was lost in bleaching (31%) and deodorizing (25%) processes.

On the other hand, TPC in crude rapeseed oils (14.9–37.4 mg CA/100 g) and extra virgin olive oils (13.1–56.7 mg CA/100 g) had higher values than deodorized rapeseed oils (4.57 and 18.0 mg CA/100 g for the pressed and the extracted oils, respectively) and refined olive oil (1.88 mg CA/100 g) (Tables 2, 3). For comparison, the average concentrations of phenols in the olive oils studied were similar to the reported results for extra virgin and olive oils (3.4–35.8 mg/100 g [1], 1.4–26.5 mg/100 g [4], 13.9–34.0 mg/100 g [9], 13.5–44.0 mg/100 g [20]).

Moreover the extracted rapeseed oils (16.7–37.4 mg CA/100 g) are a richer source of phenolic compounds than the pressed rapeseed oils (4.57–22.9 mg CA/100 g). The amounts of TPC determined in the refined pressed and extracted rapeseed oils were about 10 and 45 times higher, than obtained by Koski et al. (0.3–0.4 mg CA/100 g) [20]. It is notable that the refining process caused ca. 80 and 30% decrease of TPC in pressed and extracted rapeseed oils, respectively (Fig. 1). The neutralization step of the conventional refining process removed the highest amounts of phenolics from pressed rapeseed oil (40%). Similar decreases in TPC in soybean oil after degumming and neutralization (64%) processes were observed by Kania et al. [2].

Determination of the Antioxidant Capacity of Rapeseed and Olive Oils

The AC of rapeseed oils at various stages of the refining process and olive oils were determined by FRAP and ORAC methods and the results presented in Tables 2 and 3. It is evident that the FRAP results and ORAC values for each of the oil sample are significantly different from one

Table 2 Fatty acid composition (wt.%), tocopherols, total phenolics content (TPC), ORAC and FRAP values of the studied rapeseed oils

Compound	Pressed rapeseed oils samples						Extracted rapeseed oils samples			
	1 crude	2 crude	3 degummed	4 neutralized	5 bleached	6 deodorized	7 crude	8 crude	9 bleached	10 deodorized
C16:0	4.4	4.4	4.4	4.6	4.4	4.4	4.5	4.5	4.5	4.5
C16:1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.2
C18:0	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
C18:1 *cis*	61.1	61.5	61.5	61.1	61.2	61.2	60.3	60.7	60.3	60.7
C18:2 *trans*	0.05	–	–	–	–	0.05	0.05	–	0.05	–
C18:2 *cis*	19.2	18.6	18.6	19.4	19.2	19.2	19.8	19.3	19.8	19.6
C18:3 *trans*	0.07	0.09	0.14	–	0.13	0.44	0.12	0.06	0.13	0.38
C18:3 *cis*	9.5	9.3	9.3	9.5	9.5	9.0	9.6	9.3	9.5	9.1
C20:0	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
C20:1	1.6	1.8	1.8	1.7	1.6	1.6	1.6	1.8	1.6	1.7
C22:0	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
C22:1	0.8	1.1	1.0	0.7	0.7	0.7	0.7	1.0	0.7	0.8
C24:0	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1
C24:1	0.2	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.2	0.2
ΣSAFA	7.2	7.2	7.2	7.4	7.2	7.3	7.3	7.3	7.3	7.3
ΣMUFA	63.9	64.8	64.7	63.7	63.8	63.9	63.0	64.0	63.1	63.6
ΣPUFA	28.8	28.0	28.0	28.9	28.8	28.7	29.6	28.7	29.5	29.1
MUFA/PUFA	2.2	2.3	2.3	2.2	2.2	2.2	2.1	2.2	2.1	2.2
α-tocopherol (mg/kg)	180	230	220	230	250	240	240	300	230	290
β-tocopherol (mg/kg)	–	–	–	–	–	–	–	–	–	–
γ-tocopherol (mg/kg)	370	360	350	360	370	340	390	380	400	370
δ-tocopherol (mg/kg)	5	10	10	10	10	10	10	10	10	10
TTC (mg/kg)	555	600	580	600	630	590	640	690	640	670
TPC ± SD (mg CA/100 g)[a]	22.9 ± 0.54	14.9 ± 0.76	20.0 ± 0.48	10.8 ± 0.23	7.70 ± 0.16	4.57 ± 0.27	25.9 ± 1.2	37.4 ± 0.78	16.7 ± 0.21	18.0 ± 0.70
ORAC ± SD (μmol TE/100 g)[a]	682 ± 6.89	640 ± 28.0	605 ± 14.5	336 ± 7.21	238 ± 6.18	160 ± 5.66	994 ± 8.95	1106 ± 15.5	302 ± 8.41	296 ± 7.28
FRAP ± SD (μmol TE/100 g)[a]	295 ± 4.86	284 ± 2.63	280 ± 3.91	154 ± 2.07	112 ± 3.05	95.6 ± 2.00	465 ± 9.61	552 ± 3.82	250 ± 7.00	186 ± 4.37

SD standard deviation, *TTC* total tocopherols content, *CA* caffeic acid, *TE* trolox

[a] $n = 5$

Table 3 Fatty acid composition (wt.%), tocopherols, total phenolics content (TPC), ORAC and FRAP values of the studied olive oils

Compound	Extra virgin olive oils samples				Composed olive oils samples				Refined olive oil
	1	2	3	4	5	6	7	8	9
C16:0	12.4	11.8	10.3	14.3	9.9	10.6	11.4	14.5	10.3
C16:1	0.9	0.9	0.7	1.5	0.6	0.8	0.9	1.0	0.7
C18:0	3.7	4.0	3.5	2.8	3.5	3.4	3.8	2.6	3.4
C18:1 *cis*	77.1	77.5	75.2	70.7	76.4	75.1	77.1	71.6	75.0
C18:2 *trans*	–	–	0.05	–	–	–	0.05	0.12	0.05
C18:2 *cis*	4.7	4.5	6.6	9.6	7.7	7.8	5.4	9.1	8.3
C18:3 *trans*	–	–	–	–	0.07	0.32	0.05	–	0.28
C18:3 *cis*	0.7	0.7	1.4	0.6	0.8	0.7	0.7	0.5	0.7
C20:0	0.3	0.3	0.3	0.3	0.5	0.6	0.3	0.3	0.5
C20:1	0.2	0.2	0.7	0.2	0.3	0.4	0.2	0.2	0.3
C22:0	–	–	0.1	–	0.1	0.1	–	–	0.2
C22:1	–	–	–	–	–	–	–	–	–
C24:0	–	–	–	–	0.1	0.1	–	–	0.2
C24:1	–	–	–	–	–	–	–	–	–
ΣSAFA	16.4	16.1	14.2	17.4	14.1	14.8	15.5	17.4	14.6
ΣMUFA	78.2	78.6	76.6	72.4	77.3	76.3	78.2	72.8	76.0
ΣPUFA	5.4	5.2	8.1	10.2	8.6	8.8	6.2	9.7	9.3
MUFA/PUFA	14.5	15.1	9.5	7.1	9.0	8.7	12.6	7.5	8.2
α-tocopherol (mg/kg)	130	160	160	190	170	170	160	80	170
β-tocopherol (mg/kg)	–	–	–	–	–	–	–	–	–
γ-tocopherol (mg/kg)	–	–	–	–	–	–	–	–	–
δ-tocopherol (mg/kg)	–	–	–	–	–	–	–	–	–
TTC (mg/kg)	130	160	160	190	170	170	160	80	170
TPC ± SD (mg CA/100 g)[a]	56.7 ± 0.79	13.1 ± 0.47	33.3 ± 0.67	19.3 ± 0.23	16.6 ± 0.28	16.2 ± 0.67	24.1 ± 0.30	6.65 ± 0.32	1.88 ± 0.07
ORAC ± SD (μmol TE/100 g)[a]	902 ± 4.45	433 ± 7.77	949 ± 5.55	537 ± 13.1	590 ± 4.24	586 ± 8.82	648 ± 7.22	183 ± 6.86	123 ± 5.03
FRAP ± SD (μmol TE/100 g)[a]	151 ± 3.33	61.6 ± 2.01	167 ± 1.69	107 ± 0.60	61.8 ± 1.46	87.3 ± 1.58	103 ± 3.82	38.3 ± 1.10	32.1 ± 0.63

SD standard deviation, *TTC* total tocopherols content, *CA* caffeic acid, *TE* trolox

[a] $n = 5$

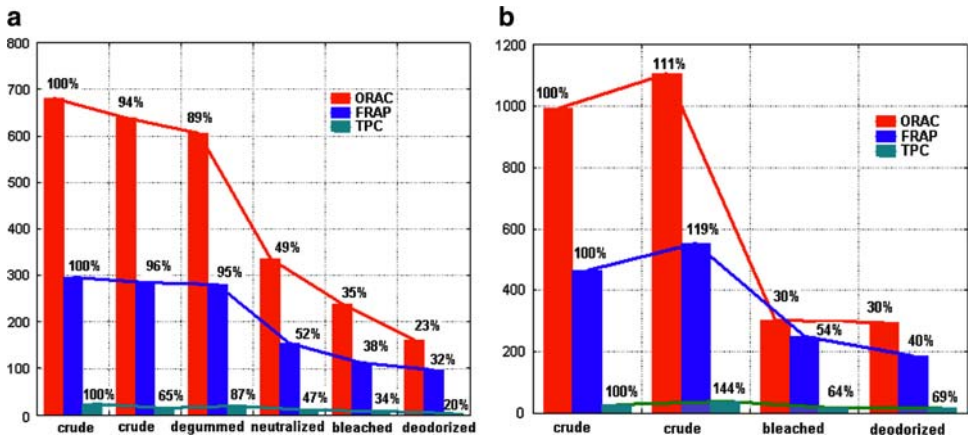

Fig. 1 Influence of refining process on TPC, FRAP and ORAC results for the pressed rapeseed oils (**a**) and the extracted rapeseed oils (**b**)

another. This variability among the same oils can be explained by the influences of genetic, environmental and technological factors, which would affect the level of antioxidants content. It is noteworthy that FRAP values were about 2 and 6 times lower in comparison with ORAC results for rapeseed oils and olive oils, respectively. This fact can be explained, that FRAP results reflect only the antioxidant reducing potential based on ferric ion, instead of the antioxidant preventive effect, whereas ORAC assay directly measures the antioxidant activities of chain-breaking antioxidants against peroxyl radicals. Furthermore, the FRAP values for all the olive oils studied (32.1–167 μmol TE/100 g) (Table 3) were lower in comparison to all rapeseed oils (95.6–552 μmol TE/100 g) (Table 2). Probably, there are some antioxidants in olive oils which do react with $Fe(TPTZ)_2(III)$ slower than antioxidants in rapeseed oils [14]. As can be seen in Table 2, the AC of the extracted rapeseed oils (ORAC values 296–1,106 μmol TE/100 g and FRAP values 186–552 μmol TE/100 g) were higher than the AC of the pressed rapeseed oils (ORAC values 160–682 and FRAP values 95.6–295 μmol TE/100 g). Only, Hay et al. [10] used the ORAC method for determination of the AC of cold pressed rapeseed oil (canola) and obtained similar result $ORAC_{oil}$ = 297 μmol TE/100 g. Moreover, the ORAC and FRAP results obtained for the rapeseed oils studied indicated that refining process caused a 80–60% decrease in their antioxidant capacities (Fig. 1). It is noteworthy that, the highest decrease in the AC determined by ORAC (40%) and FRAP (43%) took place during the neutralization of the pressed rapeseed oil. Also, 40% of TPC was removed after this step of the conventional refining process. Phenolics are polar compounds and many of them are weak acids, so they can easily be removed from the oils with aqueous solutions, especially when neutralized with sodium hydroxide. After the neutralization, phenolic components markedly partitioned toward the water phase entrapped in the sodium soap aggregates [21, 22]. Therefore, TPC and AC of rapeseed oil, subsequent to the neutralization, significantly decreased.

However, only two extra virgin olive oils presented high antioxidant capacities (902 and 949 μmol TE/100 g for the ORAC method, 151 and 167 μmol TE/100 g for the FRAP method), whereas the AC for two other extra virgin olive oils were significantly smaller (433 and 537 μmol TE/100 g, 61.6 and 107 μmol TE/100 g for ORAC and FRAP methods) (Table 3). Thus, AC values for these extra virgin olive oils were comparable to the AC of olive oils composed of refined olive oil and virgin olive oil (586–648 μmol TE/100 g for ORAC and 61.8–103 μmol TE/100 g for FRAP assays), although higher than for refined olive oil (ORAC 123 μmol TE/100 g and FRAP 32.1 μmol TE/100 g) (Table 3). For comparison, the ORAC values for olive oils, reported by others, were in the same range (155–700 μmol TE/100 g) [8–10]. In addition, in the work of Manna et al. [1], the antioxidant capacities of olives measured by FRAP method were at the same levels (22–167 μmol Fe(II)/100 g).

The within-day precision of ORAC and FRAP methods were tested by analyses of all oils in five replicates. The values of RSD ranged between 0.49–4.37 and 0.56–3.71% for AC determination by the ORAC and FRAP methods, indicating reasonable repeatability of the methods used. In comparison, Ninfali et al. [8] and Hay et al. [10] obtained higher relative standard deviations (4.17–8.98 and 11.34%) for AC determined in olive oils and rapeseed oils by the ORAC method. Also, Manna et al. [1] found a somewhat higher values of RSD = 5% for AC of olives analysis by the FRAP method.

Correlation and Principal Component Analysis

The results of AC determination by two different methods indicated that, there is a significant correlation between FRAP and ORAC values for the rapeseed oils studied (r = 0.9665, P = 0.000005) and olive oils (r = 0.9298,

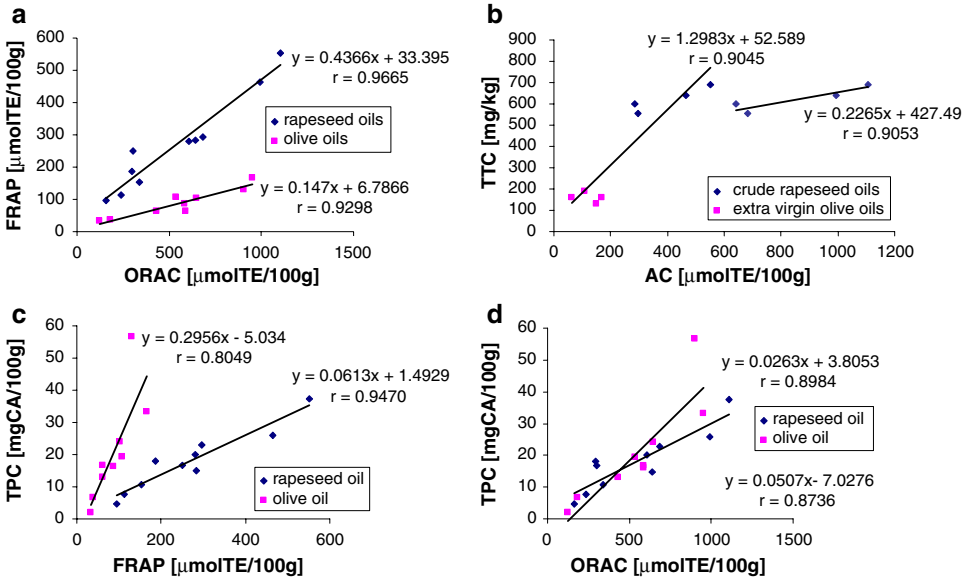

Fig. 2 Correlation between: FRAP and ORAC methods (**a**), total tocopherols content (TTC) and antioxidant capacities (AC) of oils (**b**), total phenolics content (TPC) and FRAP values (**c**) and total phenolics content (TPC) and ORAC values (**d**)

$P = 0.000282$) (Fig. 2a). Besides, the antioxidant capacity data obtained by the FRAP procedure were highly correlated with the total phenolics content in rapeseed oils ($r = 0.9470$, $P = 0.000032$) and in olive oils ($r = 0.8049$, $P = 0.008873$) (Fig. 2c). However, a somewhat higher correlation coefficient ($r = 0.9898$, $P < 0.0002$) for the same relationship was reported by Manna et al. [1]. A similar correlation between ORAC values and TPC for rapeseed ($r = 0.8984$, $P = 0.000412$) and olive oils ($r = 0.8736$, $P = 0.00208$) was observed (Fig. 2d). Also, significant correlations between ORAC values and total phenols in extra virgin olive oils ($r = 0.825$, $P < 0.001$) were calculated by Ninfali et al. [9]. However, results of antioxidant capacities and total tocopherols content (TTC) for all the oils studied do not correlate well. For comparison, Hay et al. [10] did not find a linear correlation between total tocopherol contents and the antioxidant capacity (by ORAC $r^2 = 0.375$). On the other hand, there is a significant, linear relationship between TTC and FRAP results for crude rapeseed oils and extra virgin olive oils ($r = 0.9045$, $P = 0.002023$) (Fig. 2b). Moreover, it was found linear but not significant correlation between total tocopherol contents in crude rapeseed oils and the ORAC values ($r = 0.9053$, $P = 0.09470$) (Fig. 2b).

Principal component analysis was applied to observe any possible clusters within the analyzed samples. The first two principal components took into account 94.73% (PC1 = 62.99% and PC2 = 31.74%, respectively), of the total variation. The scores of the first two principal components, for ten rapeseed oils and nine olive oils are presented in Fig. 3. In the score plot, rapeseed oils were located on the right, whereas olive oils were situated on the left in the diagram. The rapeseed and olive oils studied fell into three distinct groups, respectively. These oils groups generally have similar antioxidant capacities. The neutralized (4), bleached (5) and deodorized (6) pressed rapeseed oils were clustered with bleached (9) and deodorized (10) extracted rapeseed oils and located in the same quarter of the PCA graph. However, crude extracted rapeseed oils (7 and 8) with high antioxidant capacities were separated from crude (1 and 2) and degummed (3) pressed rapeseed oils (AC lower about two times). It is noteworthy that, two extra virgin olive oils (1 and 3) formed a cluster separated from the group including other extra virgin olives (2 and 4) and three blended olive oils samples (5, 6 and 7). Moreover, composed olive oil sample (8) and refined olive oil (9) created evidently distinct cluster. Principal component analysis graph revealed, that the studied oils with high antioxidant capacities (unrefined rapeseed oils, extra virgin

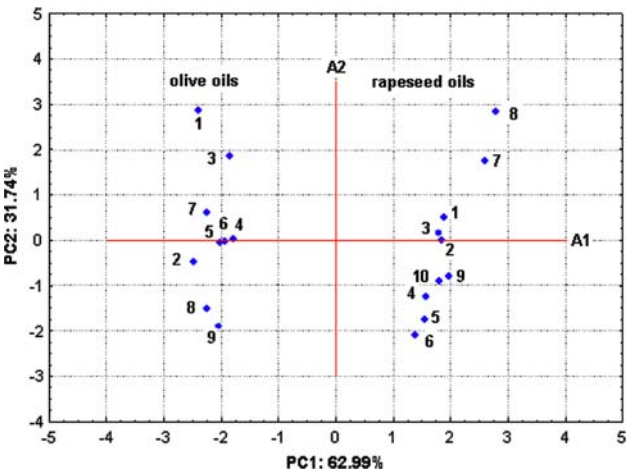

Fig. 3 Principal component analysis plot of data from antioxidant capacity determination, total phenolics and tocopherols contents and fatty acid composition of rapeseed and olive oils

olive oils except sample 2, and blended olive oils except sample 8) were situated in the upper side of the scores plot, whereas oils with lower FRAP and ORAC values [rapeseed oils after different steps of refining, extra virgin olive oil (2) blended olive oil (8) and refined olive oil (9)] are located under the A1 axis.

The proposed ORAC and FRAP methods are relatively simple, precise and convenient for the determination of antioxidant capacities of rapeseed and olive oils. It is noteworthy that, there is linear and significant correlation between these two different methods. The studied crude rapeseed oils are rich in antioxidants. The unrefined rapeseed oils have similar or higher antioxidant capacities than extra virgin olives. However, the refining processes caused the decreasing of antioxidant capacity and contents of polyphenols and tocopherols in rapeseed oils. Nevertheless, the ORAC and FRAP results for the rapeseed and olive oils analyzed correlate significantly with the total phenolics and tocopherols contents of these oils. Therefore the proposed ORAC and FRAP methods can be usefully employed by the oil processing industry in assessing of antioxidant capacities of oils and the modification of the refining process.

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