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Comparison of different tests used in mapping the Greek virgin olive oil production for the determination of its total antioxidant capacity

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RESUMEN

Comparación de diferentes test para la determinación de la capacidad antioxidante total en el mapeo de la producción de aceite de oliva virgen Griego

El objetivo de este estudio es el mapeo de la actividad antioxidante total (TAC) de 50 aceites de oliva Griego de los años 2005-2006 de acuerdo a su región y cultivar, y se comparan los ensayos del ácido 2, 2'-azino-bis (3-etilbenzo-tiazolina-6-sulfónico (ABTS), del 2,2-difenil-1-picrlhidrazil radical (DPPH) y de Folin-Ciocalteu. La capacidad antioxidante determinada en la fracción hidrofílica varió entre 5.42-22.5 mM de ácido gálico Kg^{-1} de aceite para el método ABTS y 1.29-9.95 mM Kg^{-1} de aceite para el método de DPPH mientras que la TAC del aceite de oliva completo varió entre 77-177 mM Kg^{-1} de aceite por el método de DPPH. Los resultados del contenido de fenoles totales varió entre 3.8 y 29.4 mM Kg^{-1} de aceite. El contenido total de fenoles correlaciona con la capacidad total antioxidante evaluada en la fracción hidrofílica en los ensayos de DPPH ($r = 0.89$) y de ABTS ($r = 0.69$). Los valores de DPPH de la fracción hidrofílica correlacionan significativamente con los valores de ABTS ($r = 0.81$). Sin embargo, los valores de DPPH para el aceite de oliva completo correlacionan pobremente con el ensayo ABTS, el método de Folin-Ciocalteu y el ensayo de DPPH de la fracción hidrofílica. Aunque el contenido de fenoles totales muestra una buena correlación con los valores de ABTS y DPPH y podría servir como un útil indicador de la capacidad antioxidante del aceite de oliva, el uso de una batería de test contribuye a una mejor caracterización de la capacidad antioxidante del aceite de oliva.

PALABRAS CLAVE: ABTS – Aceite de oliva – Antioxidantes fenólicos – Capacidad antioxidante total – DPPH – Folin-Ciocalteu

SUMMARY

Comparison of different tests used in mapping the Greek virgin olive oil production for the determination of its total antioxidant capacity

This study aims to map the total antioxidant capacity (TAC) of 50 Greek olive oil samples from the 2005-2006 season according to production region and cultivar and to compare the 2, 2'-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid (ABTS), 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) and Folin-Ciocalteu tests for use with olive oil. Antioxidant capacities determined in the hydrophilic fraction range between 5.42 - 22.5 mM gallic acid Kg^{-1} olive oil for the ABTS method and 1.29 - 9.95 mM Kg^{-1} for the DPPH method while in total, olive oil TAC ranges between 77 - 177 mM Kg^{-1} as assessed by the DPPH method. The results of total phenol content range between 3.8 and 29.4 mM Kg^{-1} olive oil. Total phenol content correlates with total antioxidant capacity assessed in the hydrophilic fraction through the DPPH ($r = 0.89$) and the ABTS ($r = 0.69$) assays. The hydrophilic fraction DPPH values correlate significantly with the ABTS values ($r = 0.81$). However, the DPPH values for total olive oil correlate poorly with the ABTS assay, the Folin-Ciocalteu method and the DPPH assay in hydrophilic fraction. Although total phenolic content shows good correlation with ABTS and DPPH values and could serve as a useful indicator for olive oil antioxidant capacity, the use of a battery of tests contributes to better characterization of the antioxidant capacity of olive oil.

KEY-WORDS: ABTS – DPPH – Folin-Ciocalteu – Olive oil – Phenolic antioxidants – Total antioxidant capacity.

1. INTRODUCTION

Olive oil, which is the main lipid source in the Mediterranean diet, is obtained from the fruit of several cultivars of the olive tree *Olea europea* L.. Each cultivar exhibits specific physical and biochemical characteristics, providing oils with different compositions and properties. The composition of olive oil, and its sensorial characteristics, besides being strongly dependent on the cultivar, is also influenced by several other factors like climatic and agronomic conditions, the time of harvest and agricultural practices. Extra virgin olive oil is a rich source of natural antioxidants such as tocopherols, carotenoids, sterols and phenolic compounds. Studies indicate that these phytochemicals, especially polyphenols, have high free-radical scavenging activity, which helps reduce the risk of chronic diseases, such as cardiovascular disease, cancer and age-related neuronal degeneration (Mascitelli *et al.*, 2007). Free radicals are generated in the human body through aerobic respiration and exist in different forms, including superoxide, hydroxyl, hydroperoxyl, peroxyl and alkoxyl radicals. Natural antioxidant enzymes in healthy individuals remove these free radicals while dietary antioxidants assist the body in neutralizing free radicals. Therefore, it is important to consume foods with high contents of antioxidants, such as virgin olive oil, to reduce the harmful effects of oxidative stress.

Data on the antioxidant content of olive oil is very important for food scientists, doctors, industries and consumers. Although various methodologies have been developed for the quantitative assessment of different antioxidant compounds in olive oil, the use of a single index to characterize the antioxidant potential of olive oil is beneficial (Tuberoso *et al.*, 2007). For this, several analytical methods have been developed for olive oil total antioxidant capacity (TAC) assessment. A number of these methods measure the inhibition of a stable or an artificially generated radical upon olive oil addition. The DPPH radical scavenging assay is the commonly used method for olive oil TAC estimation and is based on the disappearance of the purple color of the radical solution, through scavenging reactions with antioxidants, measured spectrophotometrically at 515 nm. This method has been developed for both the hydrophilic (Espin *et al.*, 2000; Gorinstein *et al.*, 2003; Valavanidis *et al.*, 2004) and lipidic (Espin *et al.*, 2000) fraction. In polar fractions, the ABTS radical scavenging assay has also been used (Mannimo *et al.*, 1999; Pellegrini *et al.*, 2001; Gorinstein *et al*, 2003; Bendini *et al.*, 2006). The ABTS assay is based on the disappearance of the blue-green color of the radical solution, upon consumption by antioxidants, measured spectrophotometrically at 734 nm. Another index related to olive oil TAC is total phenolic content as measured through the Folin-Ciocalteu assay (Montedoro *et al.*, 1992; Capannesi *et al.*, 2000). Olive oil total phenolics are determined spectrophotometrically at 765 nm in methanolic extracts.

This work aims to map the antioxidant capacity of Greek olive oils according to production region and cultivar. Furthermore, the effect of cultivar and region on results is indicated and the ABTS, DPPH and Folin-Ciocalteu assays are compared during the analysis of the 2005-2006 harvest.

2. MATERIALS AND METHODS

2.1. Chemicals

Gallic acid monohydrate was supplied from Riedel-de Haën. 2, 2-Diphenyl-1-picrylhydrazyl radical (DPPH), of 90% grade, 2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), of 98% grade, and peroxidase (HRP), type II, 222 U mg^{-1}, were obtained from Sigma. Ethyl acetate and methanol of analytical grade were obtained from Merck and n-hexane, of 95% grade, was from Lab Scan. Hydrogen peroxide, 30% in water solution, of analytical grade, sodium acetate, of pro analysis grade, and Folin-Ciocalteu reagent were purchased from Merck. Sodium carbonate was obtained from SDS. Distilled water was used throughout.

2.2. Apparatus

Experiments for ABTS, DPPH and Folin-Ciocalteu assays were performed with a double beam Jasco V-550 UV-Vis spectrophotometer. An Orion® pH-meter was used to prepare aqueous acetate and carbonate buffers.

2.3. Olive oil samples

Fifty extra virgin olive oil samples originating from Messinia, Zakinthos, Chalkidiki, Iraklio, Chania, Lesbos, Lakonia, Pieria, Arkadia and Euvia, belonging to Koroneiki, Prasinolia, Athinolia, Adramatini, Kolovi, Mavrolia, Kolindrou, Megaron and Chalkidikis cultivars from Greece were stored at −80 °C and protected from light until analysis. Samples came from the 2005-2006 harvest. Samples had a fully characterized profile, according to acidity % oleic acid, peroxide number, Rancimat value, K_{232}, K_{270}, total sterols, % content in erythrodiol-oubaol, cholysterol, brassicasterol, campesterol, b-sitosterol, D-7 stigmasterol, % content in fatty acids C16:0, C16:1, C17:0, C17:1, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1, C22:0.

2.4. Hydrophilic extracts

0.50 g of olive oil sample was diluted 1:1 (v:v) in n-hexane. Diluted samples were extracted by two 0.50 mL portions of methanol:water 80:20 (v:v) solvent. After separation from the lipidic fraction by 5 min of centrifugation at 5000 rpm, the two hydrophilic extracts were combined.

2.5. DPPH assay: total olive oil and hydrophilic fraction

A 1.3×10^{-4} M working solution of the DPPH radical in ethyl acetate which shows an absorbance of approximately 1.2 at 515 nm was prepared daily. Olive oil aliquots of 20, 80, 120 and 180 mg or 170 μL of olive oil hydrophilic extract were added to 4.0 mL DPPH working solution. Mixtures were vigorously stirred for a few seconds and kept in the dark for 1 h. Absorbencies were measured at 515 nm against ethyl acetate. Olive oil antioxidants scavenge the DPPH cation radical, resulting in decolorization of its purple solution. Total antioxidant capacity, expressed as mmol L^{-1} of gallic acid equivalents (GAE) per kilogram of oil, is calculated using the appropriate amount that shows 50% absorbance inhibition as determined by plotting absorbencies against the amount of olive oil .

2.6. ABTS assay in the hydrophilic fraction

The ABTS method as described by Pellegrini *et al.* (2001) was modified by changing the oxidation reagent from potassium persulfate to hydrogen peroxide using HRP catalyst: ABTS, H_2O_2 and HRP stock solutions at concentrations of 20, 20 mM and 55.5 IU mL^{-1}, respectively, were prepared in 0.020 M acetate buffer, pH 4.6 and were stable for over a month stored at 0-4 °C. An ABTS radical cation working solution was prepared by mixing 16, 0.16 and 5.4 mL of the three stock solutions, respectively

in a 100 mL volumetric flask. The formation of ABTS radical cation is completed within 3 h in the dark. A fifty fold excess of ABTS over H_2O_2 was chosen in order to prevent possible reactions between the antioxidants and unreacted hydrogen peroxide. It should be noted that the presence of ABTS is necessary to stabilize the ABTS radical cation (Labrinea and Georgiou, 2005). After reaction completion, the volumetric flask is filled up with methanol. The thus prepared working ABTS radical cation solution contains 1.6 mM ABTS and 1.6 mM $ABTS^{+\bullet}$ and shows an absorbance of approximately 1.2 at 734 nm. 20, 100, 180 and 260 µL of hydrophilic extract were added to 4.0 mL of working solution and brought to 5.0 mL final volume with methanol:water 80:20 (v:v). Mixtures were vigorously stirred for a few seconds and kept in the dark for 1 h. Absorbencies were measured at 734 nm against methanol:water 80:20 (v:v). Olive oil antioxidants scavenge the ABTS cation radical, resulting in its decolorization. Total antioxidant capacity, expressed as mmol L^{-1} of gallic acid equivalents (GAE) per kilogram of oil, is calculated using the appropriate volume that shows 50% absorbance inhibition as determined by plotting absorbencies against the hydrophilic extract volumes.

2.7. Total phenol content

Total phenol content was measured using the Folin-Ciocalteu method as described by Capannesi et al. (2000). A calibration curve of gallic acid was acquired in the concentration range of 1-50 µM.

3. RESULTS AND DISCUSSION

3.1. Determination of antioxidant capacity

The gallic acid calibration curves used for transforming absorbance inhibition values (AI) to gallic acid equivalents (GAE, µM), for ABTS and DPPH assays, are: $AI_{ABTS} \times 10^2 = (6.10 \pm 0.71) \times GAE + (6.2 \pm 4.0)$, $r = 0.997$ and $AI_{DPPH} \times 10^2 = (6.78 \pm 0.31) \times GAE + (1.4 \pm 2.6)$, $r = 0.998$. The concentration range is 2-50 µM.

A linear correlation between the absorbance inhibition of the DPPH or ABTS radical solutions and the mass of both hydrophilic extract and olive oil sample was observed for all analyzed olive oil samples. Figure 1 shows this linear correlation, for three olive oil samples with high, middle and low TAC values. A higher slope indicates higher antioxidant activity. The linear dose response relation justifies the use of extrapolation for determining IC50% concentrations. In this paper all results are per kg olive oil.

Antioxidant capacities of the analyzed extra virgin olive oil samples measured by ABTS and DPPH assays are shown in Table 1. Olive oil samples are from different cultivars and origins. Antioxidant capacities determined in the hydrophilic fraction range between 5.42 - 22.5 and 1.29 - 9.95 mM gallic acid Kg^{-1} olive oil for ABTS and DPPH methods, respectively. For total olive oil, TAC ranges between 77 and 177 mM gallic acid Kg^{-1} measured by the DPPH method. Results from the DPPH assay in total olive oil are higher than those in the hydrophilic fraction. This is expected as the DPPH assay in total olive oil also assesses tocopherols which show a synergistic action with phenolics (Espin *et al.*, 2000). The mean TAC values for ABTS and DPPH in the hydrophilic fraction are 13.1 ± 4.2 and 5.4 ± 2.2 mM gallic acid Kg^{-1}, respectively. It is interesting to note that for the hydrophilic fraction, the DPPH test gives systematically lower results than the ABTS assay. This is probably due to the different reaction properties of the olive oil antioxidants extracted in the hydrophilic fraction.

%RSD values for the DPPH assay in total olive oil were in the range of 2.2% to 3.5%, while for the

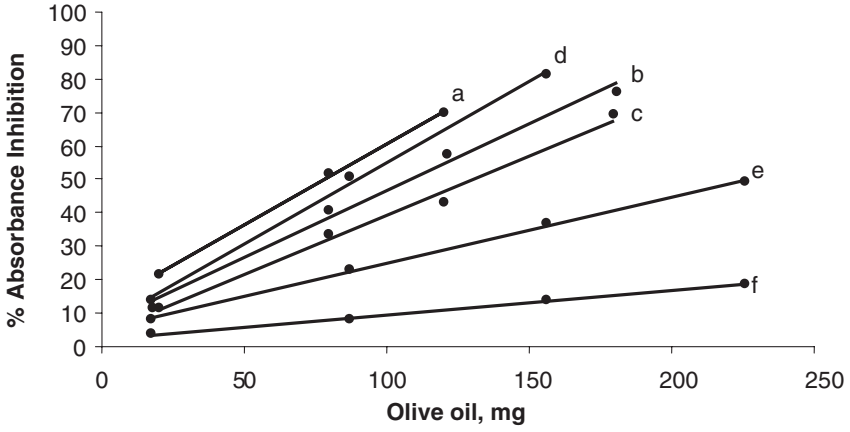

Figure 1
Relationship between the amount of olive oil sample and % absorbance inhibition as measured through the DPPH (a, b, c) and ABTS (d, e, f) assays for three olive oil samples with high (a & d); middle (b & e); and low (c & f) TAC values. Slopes ± SD and correlation coefficients where: (a) 7.73 ± 0.20, 0.9997; (b) 6.39 ± 0.44, 0.995; (c) 5.66 ± 0.39, 0.995; (d) 7.77 ± 0.41, 0.998; (e) 3.149 ± 0.094, 0.9991; (f) 1.159 ± 0.046, 0.998.

Table 1
Total Antioxidant Capacity and Total phenolics of different Greek cultivar-origin olive oils determined through DPPH, ABTS and Folin-Ciocalteu assays

			Phenolics[a]	TAC[a]		
				ABTS	DPPH	DPPH
Sample No	Origin	Cultivar	Folin-Ciocalteu	Hydrophilic fraction		Total olive oil
1	Chania	Koroneiki	12.3	8.65	3.67	90.5
2	-//-	-//-	14.3	8.51	4.03	95.1
3	-//-	-//-	24.9	14.1	7.10	122
4	-//-	-//-	10.3	11.8	3.95	79
5	-//-	-//-	15.6	14.8	5.22	96
6	-//-	-//-	16.6	19.2	7.32	101
7	-//-	-//-	19.2	18.4	6.24	113
8	-//-	-//-	10.1	11.1	4.03	85
9	Messinia	-//-	10.0	5.71	3.06	90.2
10	-//-	-//-	7.8	5.42	2.61	83.4
11	-//-	-//-	12.1	8.23	4.10	126
12	-//-	-//-	17.0	8.77	5.35	98.3
13	-//-	-//-	10.2	6.86	2.44	96.3
14	-//-	-//-	14.4	16.1	5.92	117
15	-//-	-//-	15.3	14.7	4.86	93
16	-//-	-//-	3.8	7.28	1.29	77
17	-//-	-//-	15.3	16.5	4.51	96
18	-//-	Mavrolia	15.3	13.7	6.23	123
19	-//-	-//-	11.0	14.3	5.13	125
20	Lakonia	Mixed	11.7	13.7	5.92	111
21	-//-	Koroneiki	21.1	12.1	6.44	114
22	-//-	Athinolia	9.4	6.48	2.84	125
23	-//-	-//-	29.4	18.6	7.51	117
24	-//-	-//-	25.1	22.5	9.57	115
25	-//-	-//-	9.0	11.1	2.94	77
26	-//-	-//-	10.8	13.7	3.13	93
27	-//-	-//-	15.2	14.5	5.61	129
28	Zakinthos	Koroneiki	13.2	10.8	5.91	122
29	-//-	-//-	24.7	17.4	9.12	119
30	-//-	-//-	19.6	13.2	6.95	117
31	-//-	-//-	22.9	17.0	9.20	118
32	-//-	-//-	8.1	10.9	3.09	96
33	-//-	-//-	7.6	11.4	3.25	86
34	-//-	-//-	14.0	13.7	4.90	99
35	Euvoia	Megaron	7.5	10.7	2.04	78
36	Pieria	-//-	11.4	8.21	3.88	175
37	-//-	Kolindrou	20.3	11.7	5.79	112
38	Chalkidiki	Chalkidikis	26.2	22.1	9.95	110
39	-//-	-//-	16.0	17.1	5.58	94
40	-//-	Prasinolia	21.5	8.75	5.16	127
41	-//-	-//-	13.8	7.01	3.28	82.6
42	Arkadia	Athinolia	15.9	15.8	6.30	96
43	Iraklio	Koroneiki	24.1	15.3	8.72	131
44	-//-	-//-	24.2	15.5	6.26	167
45	-//-	-//-	28.0	17.3	8.71	133
46	-//-	-//-	21.9	17.7	8.92	148
47	-//-	-//-	12.0	11.9	3.21	88
48	Lesbos	Adramatini	27.8	18.4	9.69	119
49	-//-	Kolovi	17.4	11.4	4.28	177
50	-//-	-//-	12.3	14.3	4.36	90

[a]mM gallic acid equivalents kg^{-1} olive oil

hydrophilic fraction, they were between 7% and 9.9%. For the ABTS method %RSD values range between 6.9% and 9.7%. From these results it is clear that precision is lower for the hydrophilic extracts due to the extraction step.

3.2. Total phenol content

The total phenol content (TPC) was expressed as mmol L^{-1} of gallic acid equivalents (GAE) per kilogram of olive oil, using the equation TPC x 10^2 = (1.938 ± 0.066) x GAE + (1.8 ± 1.7), r = 0.998. Results from the analyzed olive oil samples, presented in Table II, range between 3.8 and 29.4 mM gallic acid Kg^{-1} olive oil. The mean phenol content is 16 ± 6.3 mM gallic acid Kg^{-1}. %RSD values were in the range of 6.4% to 8.8%. Total phenolic content determined through the Folin method are in a similar range to those determined through the ABTS assay in the hydrophilic fraction.

3.3. Correlation between total phenol content and the different TAC measurement methods

It is interesting to examine the relationship between the olive oil total phenol content and the antioxidant capacity measured by different methods. In most cases, the antioxidant capacity assessed in the hydrophilic fraction increases along the phenol content (Table 1). Figure 2 shows the correlation between the TAC values assessed through ABTS assay and the total phenol content, which results in a coefficient of r = 0.69. The graph slope shows that TAC values measured by the ABTS method are 54% lower than total phenol content. The correlation between the TAC values assessed through the DPPH assay in hydrophilic fraction and the total phenol content result in a correlation coefficient of r = 0.89. The graph slope shows that TAC values measured by the DPPH method are 68% lower than total phenol content. It is clear that the Folin-Ciocalteu method gives higher results. This is in line with previous published data (Gorinstein *et al*., 2003; Sánchez *et al*., 2007). This is due to the low specificity of the Folin-Ciocalteu method, as the color reaction can occur with any oxidizable phenolic hydroxy group (Capannesi *et al*., 2000; Hrncirik *et al*., 2004). Figure 3 shows the correlation (r = 0.81) between DPPH and ABTS methods, both in the hydrophilic fraction. The graph slope shows that TAC values measured by the DPPH method are 57% lower than those measured by the ABTS method. The correlation between DPPH assay in total olive oil and the ABTS assay is poor (r = 0.22) while it is also low with the Folin-Ciocalteu method (r = 0.50) and the DPPH assay in the hydrophilic fraction (r = 0.45) (Data not shown).

3.4. Correlation between TAC, cultivar/origin and olive oil analytical parameters

The TAC values and the total phenol content of the olive oil samples were compared in groups from the same cultivar, origin or cultivar-origin together. Table 2 shows the correlation coefficients found among results. The critical values at the conventional 5% testing level along the sampling size are: 0.8783 (n = 5); 0.8114 (n = 6); 0.7545 (n = 7); 0.7067 (n = 8); 0.6664 (n = 9); 0.6020 (n = 11); 0.3610 (n = 30) (Siegel et al., 1996). For the cases that the correlation coefficients are bigger, the correlations are statistically significant, which means that there is indeed an association between the two methods compared. The DPPH assay in hydrophilic extract gave good correlations (r range between 0.72 and 0.98) with the ABTS and the Folin-Ciocalteu method for all origins, cultivars and combinations of cultivar-origin tested. On the other hand, correlations between the DPPH method in olive oil and the other three methods in hydrophilic

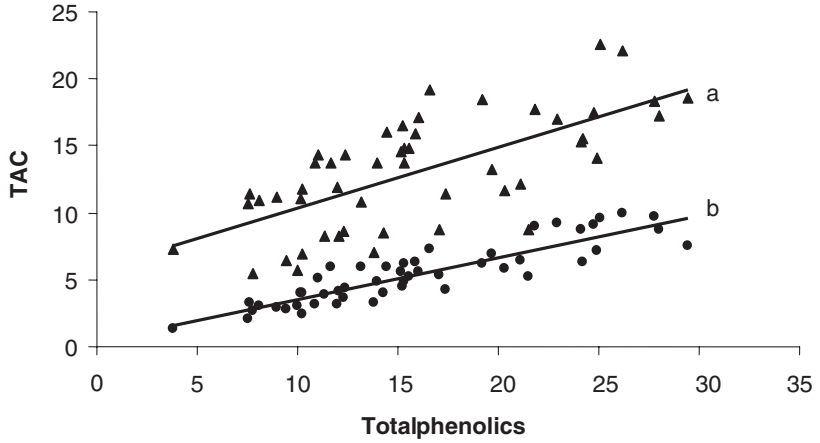

Figure 2
Correlation between extra virgin olive oil total antioxidant activity assessed in hydrophilic extracts through the ABTS (a) and DPPH (b) assays and total phenolics assessed through the Folin Ciocalteu method (n = 50). TAC values and total phenolics are expressed as mM gallic acid kg^{-1} olive oil. Correlation equations: (a) y = (0.456 ± 0.070) + (5.8 ± 1.2), r = 0.69; (b) y = (0.315 ± 0.023) + (0.37 ± 0.39), r = 0.89.

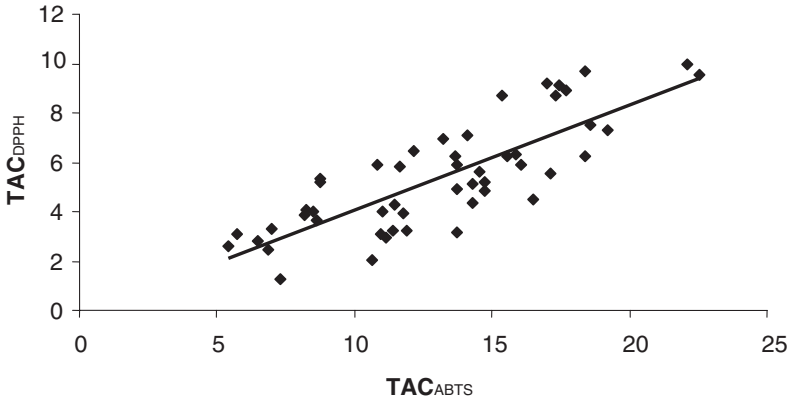

Figure 3
Total antioxidant activity of extra virgin olive oil hydrophilic extracts assessed through DPPH and ABTS assays (n = 50). TAC values are expressed in mM gallic acid kg^{-1} olive oil. Correlation equation: $y = (0.428 \pm 0.045) + (-0.22 \pm 0.62)$, $r = 0.81$.

fraction were lower (r range between 0.10 and 0.98, smaller than the corresponding critical values in most cases). Among the five origins, Lakonia shows the lowest coefficients (r range between 0.11 and 0.87). The two cultivars tested show significantly different coefficients in the correlation of DPPH in olive oil with the methods in hydrophilic fraction, where Athinolia's is systematically lower. Data was not further treated for the varieties Mavrolia, Megaron, Kolindrou, Chalkidikis, Prassinolia, Kolovi or Adramatini, which we tested using one or two olive oil samples. Although we assessed a small number of Athinolia cultivar samples, data is included as Athinolia and is a dynamic variety in Greece.

The TAC values of the 50 analyzed olive oil samples were also compared with a number of analytical parameters, mentioned in the olive oil samples section, that characterize their profile. No correlation was found between these characteristics and TAC values as determined by the four assays.

4. CONCLUSIONS

There were good correlations among the hydrophilic antioxidant activities measured by DPPH and ABTS, suggesting that these methods have a similar predictive capacity of olive oil antioxidant activity. High correlations between Folin-Ciocalteu, DPPH and ABTS methods indicate that the total phenolic content can be used as an indicator for olive oil antioxidant activity assessed in the hydrophilic

Table 2
Correlation between TAC and total phenol content results in olive oil samples classified according to origin, cultivar and origin-cultivar

Method	Folin-Ciocalteu	ABTS in extract	DPPH in extract	DPPH in olive oil
Folin-Ciocalteu		0.52 [1*]	0.83 [1]	0.98 [1]
		0.63 [2]	0.87 [2]	0.48 [2*]
	–	0.77 [3]	0.87 [3]	0.39 [3*]
		0.90 [4]	0.98 [4]	0.78 [4]
		0.83 [5*]	0.84 [5*]	0.74 [5*]
ABTS in extract	0.71 [a]		0.85 [1]	0.54 [1*]
	0.85 [b]		0.75 [2]	0.48 [2*]
	0.67 [2a]	–	0.86 [3]	0.11 [3*]
	0.85 [3b]		0.87 [4]	0.48 [4*]
			0.91 [5]	0.73 [5*]
DPPH in extract	0.92 [a]	0.79 [a]		0.81 [1]
	0.90 [b]	0.92 [b]		0.73 [2]
	0.93 [2a]	0.72 [2a]	–	0.43 [3*]
	0.92 [3b]	0.92 [3b]		0.83 [4]
				0.59 [5*]
DPPH in olive oil	0.78 [a]	0.52 [a]	0.73 [a]	
	0.39 [b*]	0.10 [b*]	0.37 [b*]	–
	0.53 [2a*]	0.35 [2a*]	0.64 [2a*]	
	0.39 [3b*]	0.13 [3b*]	0.42 [3b*]	

[1]Chania (n = 8), [2]Messinia (n = 11), [3]Lakonia (n = 8), [4]Zakinthos (n = 7), [5]Iraklio (n = 5), [a]Koroneiki (n = 30), [b]Athinolia (n = 7), [2a]Messinia-Koroneiki (n = 9), [3b]Lakonia-Athinolia (n = 6). *The computed correlation is not statistically significant and the null hypothesis that there is no correlation between methods should not be rejected.

fraction. The DPPH values of lipophilic olive oil fraction correlate poorly with the other methods.

Although the ABTS and DPPH assays gave good correlations with the Folin-Ciocalteu method, the TAC values are systematically lower. In this respect we propose that the use of this battery of tests contributes to better characterization of olive oil antioxidant capacity.

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