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Analytical Methods

High throughput flow injection bioluminometric method for olive oil antioxidant capacity

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Abstract

This paper describes a rapid flow injection automated method for the determination of olive oil total antioxidant capacity. The chemistry involved is the horseradish peroxidase (HRP) catalysed oxidation of luminol by hydrogen peroxide. Oxidation results in light emission (bioluminescence) that is enhanced using *p*-iodophenol sensitizer. Olive oil (0.7 mL) is extracted with two 0.7 mL aliquots of 80–20% (v/v) methanol–water solvent. A 17 μL aliquot of the extract containing hydrophilic antioxidants is injected in a phosphate buffer channel that subsequently merges with a luminol–HRP–*p*-iodophenol reagent stream. Bioluminescence resulting after merging the mixture with a hydrogen peroxide stream is suppressed upon increasing antioxidants' concentration resulting in negative peaks due to hydrogen peroxide consumption by antioxidants. The method has been optimized on (a) number of manifold channels, (b) flow rates, (c) coil length and (d) HRP, hydrogen peroxide and *p*-iodophenol concentrations. Detection limit is calculated at 1.5×10^{-7} M gallic acid, linear range is between 1.0×10^{-6} and 1×10^{-4} M and precision is better than 2.8% RSD ($n = 4$). The fully automated method is achieving a rate of sampling equal 180 probes per hour. The proposed method is applied for the assessment of 50 extra-virgin olive oil samples of different Greek cultivars and regions.
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Keywords: Olive oil; Total antioxidant capacity; Flow injection; Food quality control

1. Introduction

Free radicals are thought to be responsible for several pathological processes such as cancer, atherosclerosis and negative cellular changes associated with aging (Nyska & Kohen, 2002). The consumption of dietary antioxidants seems to play an important role in protecting against these degenerative events (Kaur & Kapoor, 2001) and the interest for their quantitative or qualitative determination in foods has been increased (Huang, Ou, & Prior, 2005). Olive oil contains a high amount of natural antioxidants such as tocopherols, carotenoids, sterols and phenolic compounds. Among the phenolic compounds found in extra-virgin olive oils, *o*-dihydroxy-phenolics are very potent antioxidants (Boskou, Blekas, & Tsimidou, 2005). The composition of these phenols in various olive oils is variable. The presence of antioxidants in olive oil is an important factor on the oxidative stability during storage and towards thermal degradation. A classic method for determination of olive oil polyphenol content in methanolic extracts is the Folin–Chiocalteu assay (Capannesi, Palchetti, Mascini, & Parenti, 2000; Montedoro, Servili, Baldioni, & Miniati, 1992). Polyphenol analysis is usually performed through HPLC methods resulting in the determination of specific individual antioxidant compounds (Cabrini et al., 2001; Carrasco-Pancorbo et al., 2005; Hrncirik & Fritsche, 2004; Montedoro et al., 1992). However, for olive oil quality control, it is more useful and practical to estimate total antioxidant capacity

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(TAC). This approach is more time and cost efficient and accounts for possible synergistic or antagonistic effects among antioxidants compounds. Moreover, TAC assesses recently detected antioxidant compounds in olive oil such as hydroxyl-isochromans (Bianco, Coccioli, Guiso, & Marra, 2002; Tonga, Tonga, Franconi, Marra, & Guiso, 2003). Phenolic compounds are strong free radical scavengers, so most of the analytical methods, developed for the assessment of olive oil total antioxidant capacity, measure the inhibition of an artificially generated oxidative process upon olive oil addition. These methods differ in the chemical system and the detection principle used. Other methods are based on the consumption of a stable colored free radical. The DPPH radical scavenging assay is the commonly used method for olive oil (Espin, Soler-Rivas, & Wichers, 2000; Keceli & Gordon, 2001; Rossi, Alamprese, & Ratti, 2007; Valavanidis et al., 2004). Although this assay dominates olive oil related literature, different assays based on different mechanisms are needed for complete assessment of antioxidant capacity. Along this line the ABTS and the ferric ion reducing antioxidant power (FRAP) assays have recently been used for olive oil TAC estimation (Bendini, Cerretani, Vecchi, Carrasco-Pancorbo, & Lercker, 2006; Gorinstein et al., 2003; Pellegrini et al., 2003; Pellegrini, Visioli, Buratti, & Brighenti, 2001; Saura-Calixto & Coni, 2006; Silva, Gomes, Leitao, Coelho, & Boas, 2006). As yet, just one chemiluminescence (CL) based method has been published for olive oil TAC assessment (Papadopoulos, Triantis, Yannakopoulou, Nikokavoura, & Dimotikali, 2003). Chemiluminescent reactions, thanks to advantages such as high sensitivity and selectivity, wide linear range, simplicity and the use of inexpensive instrumentation for monitoring emission, have considerable analytical potential in a great variety of applications. Automation of CL assays is beneficial concerning accuracy, reproducibility and analytical throughput. Flow injection automation of CL assays could introduce the inherent advantages of strict control of mixing and timing, improving assay reproducibility. Although FI methods with amperometric and spectrophotometric detection have been developed for olive oil TAC assessment (Battino, Politi, Bompadre, Scalzo, & Mezzetti, 2004; Mannimo, Buratti, Cosio, & Pellegrini, 1999) no CL flow injection method has been developed so far for olive oil TAC assessment.

This work presents an automated flow injection method based on a laboratory-made FI system for the determination of olive oil antioxidant capacity. The chemistry involves the horseradish peroxidase (HRP) catalyzed oxidation of luminol by hydrogen peroxide. Oxidation results in bioluminescence that is enhanced using *p*-iodophenol sensitizer (Ilyina et al., 2003). Antioxidant capacity is assessed through light emission inhibition due to hydrogen peroxide scavenging by antioxidants. The proposed method is used for the analysis of 50 olive oil samples of different Greek cultivars and geographic regions.

2. Materials and methods

2.1. Reagents and solutions

2.1.1. FI method

Hydrogen peroxide, 30% in water solution, analytical grade was obtained from Merck (Darmstadt, Germany). 3-Aminophthalhydrazide (luminol), 97%, was supplied from Aldrich (Steinheim, Germany). *p*-Iodophenol, 98+%, was from Alfa Aesar (Karlsruhe, Germany). Horseradish peroxidase (HRP) was obtained from Sigma (Steinheim, Germany). KH_2PO_4 used for buffer preparation was obtained from Panreac (Barcelona, Spain). Buffer used was 1.0×10^{-2} M KH_2PO_4, pH 7.4. Stock solutions of 20 mM luminol, 20 mM *p*-iodophenol and 80 IU mL^{-1} HRP in buffer were stable at +4 °C for one month. Mixed working solution of 2.0 mM luminol – 2.0 mM *p*-iodophenol – 0.64 IU mL^{-1} HRP and working solution of 1.0 mM H_2O_2 in buffer were prepared daily. Organic solvents, methanol, ethanol, acetonitrile, 1-propanol, 2-propanol, DMSO and acetone, of analytical grade, were obtained from Merck. Gallic acid monohydrate was supplied from Riedel-de Haën (Seelze, Germany), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), 97%, was supplied from Aldrich, 2-(4-hydroxyphenyl) ethanol (tyrosol), 98%, was from Alfa Aesar and 3,4-dihydroxybenzoic (protocatechuic) acid, of analytical grade, was obtained from Sigma. Standard solutions of gallic acid, trolox and protocatechuic acid were prepared daily in methanol:water 80:20 (v/v) in the range of 1–100 μM, and tyrosol in the range of 1–100 mM.

2.1.2. ABTS and DPPH methods

2,2′-Azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS), of 98% grade, and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), of 90% grade were obtained from Sigma. Ethyl acetate, of analytical grade, and sodium acetate, of pro analysis grade, were obtained from Merck. 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. ABTS radical cation working solution was prepared by mixing 16, 0.16 and 5.4 mL of the three stock solutions in a 100 mL volumetric flask. The formation of ABTS radical cation is completed within 3 h in the dark. After reaction completion, the volumetric flask is filled up with methanol. A 1.3×10^{-4} M working solution of the DPPH radical in ethyl acetate, that shows an absorbance of approximately 1.2 at 515 nm was prepared daily.

2.2. Sample preparation

A total of 50 extra-virgin olive oil samples of various regions-varieties from Greece were kindly provided by Minerva S.A. (Athens, Greece). Samples were stored at −80 °C until analysis. Olive oil (0.70 g) sample was extracted by two 0.70 mL portions of methanol:water

80:20 (v/v) solution and the two extracts were combined after 5 min centrifugation at 5000 rpm. For FI method, an aliquot of 1 mL extract was diluted 1:1 with the solvent.

2.3. Apparatus

The analysis is based on the laboratory-made FI analyzer depicted in Fig. 1. The analyzer consists of a Miniplus 3 Gilson peristaltic pump (Villiers, France), a VICI Valco E60-CE low pressure injection valve (Houston, TX, USA) and a straight glass tube flow cell. Light detection is through the Hammamatsu HC 135-01 photomultiplier tube (Japan) that is intergraded with analogue circuits providing RS-232 computer output. PTFE tubing, 0.8 mm i.d., was used for the construction of mixing coil of 50 cm and sample loop of 17 μL while Tygon tubing, 1.30 mm i.d., was used with the peristaltic pump. The laboratory-made software package for data acquisition and control was developed in Lab View object oriented language and provides modules for FI experiments; data treatment, measurement of peak height and correction of drifting base line; and data export to text files. Control of the pump, start and stop, and the injection valve, load and inject, are achieved through Lab View objects. The software has low system requirements and is used with a personal computer.

ABTS and DPPH methods were performed with a double beam Jasco V-550 UV–Vis spectrophotometer (Tokyo, Japan).

2.4. FI-bioluminescence measurements

A manifold of three-lines, as detailed in Fig. 1, was used. Standards and samples are injected in the buffer channel that subsequently merges with a luminol–HRP–*p*-iodophenol reagent stream. Bioluminescence resulting after merging the mixture with a hydrogen peroxide stream is suppressed upon increasing antioxidants' concentration resulting in negative peaks due to hydrogen peroxide consumption. The reagent blank is the base line signal. Flow rate was 6.3 mL min^{-1} in each channel and the laboratory temperature was kept at 25 ± 1 °C. The antioxidant capacity of the samples, expressed in mmol L^{-1} of gallic acid equivalents (GAE) per kilogram of oil, is calculated using a gallic acid calibration curve.

2.5. ABTS and DPPH assays

Hydrophilic extract (170 μL) was added to 4.0 mL of ABTS and DPPH working solutions and broad to 5 mL final volume with methanol:water 80:20 (v/v) and ethyl acetate, respectively. The spectrophotometric readings were carried out after a 1-h period of incubation, in the dark and at room temperature at 734 nm against methanol:water 80:20 (v/v) for ABTS method and at 515 nm against ethyl acetate for DPPH method. Gallic acid calibration curves in the range 2–50 μM were prepared, and data were expressed in gallic acid equivalent antioxidant capacity (GEAC, mmol L^{-1} per kilogram of olive oil).

3. Results and discussion

As 2-propanol dissolves olive oil, direct injection of olive oil sample using a different version of the flow system replacing buffer with 2-propanol and preparing reagent solution (Fig. 1) in 2-propanol was tried. Injections of spiked olive oils with trolox in the range 0.1–100 μM resulted in high negative peaks that were not related to concentration. 1/10 and 1/100 dilutions lowered peak heights but signals were again not related to concentration. This was attributed to 2-propanol use, so different solvents and solvent mixtures were tried to replace 2-propanol.

3.1. Optimization

3.1.1. Solvent selection

Seven organic solvents, methanol, ethanol, acetonitrile, DMSO, 1-propanol, 2-propanol, acetone, solvent:water mixtures of 80:20 (v/v) and 20:80 (v/v), and deionized water were injected in the FI system in order to choose the appropriate one for diluting olive oil extracts. Methanol, ethanol, DMSO, 1-propanol and acetone show negative blank peaks due to their oxidation by H_2O_2 that inhibits bioluminescence. Acetonitrile and 2-propanol

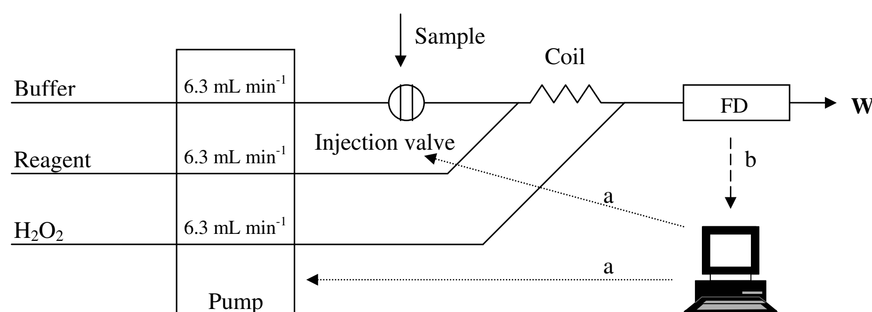

Fig. 1. Laboratory-made flow injection system for olive oil total antioxidant capacity assessment through chemiluminescence. Buffer: 1.0×10^{-2} M phosphate, pH 7.4; reagent: 2.0 mM luminol, 2.0 mM *p*-iodophenol and 0.64 IU mL^{-1} HRP in buffer; H_2O_2: 1.0 mM in buffer; sample loop: 17 μL; coil: 50 cm; FD: flow detector; W: waste; a: digital control lines; b: data acquisition line.

show positive blank peaks probably due to incomplete mixing with the buffer carrier stream. It should be noted that acetonitrile and 2-propanol have been previously used as solvents for oxidation reactions with hydrogen peroxide (Croston, Langston, Sangoi, & Santhanam, 2002; Sakharov & Skibida, 1995) that is acetonitrile and 2-propanol are not oxidized by hydrogen peroxide. All five solvents that show negative blank peaks were tried in solvent:water 80:20 (v/v) mixtures resulting in lower blank peaks. Among them methanol:water 80:20 (v/v) shows the smallest blank peak allowing lower detection limits. Although a further decrease of blank is achieved by increasing water concentration to 80%, methanol:water 80:20 (v/v) solvent was selected as it is used extensively for the extraction of phenolic antioxidants from olive oil (Angerosa, D'Alessandro, Konstantinou, & Di Giacinto, 1995; Brenes, García, García, Ríos, & Carrido, 1999; Montedoro et al., 1992). Two extraction steps are recommended in order to recover the whole amount of hydrophilic antioxidants. The combined extract was diluted 1:1 with the solvent to adjust concentration to the gallic acid calibration curve. It should be noted that methanol is oxidized by hydrogen peroxide resulting in negative blank peaks that could cover signals resulting from injection of low concentration solutions. Although this is a potential problem, our results indicate that this oxidation reaction is slow in methanol:water 80:20 (v/v) solvent. Moreover, hydrogen peroxide stream is added at a high flow rate just before the detector (Fig. 1). This way, the time allowed for the reaction to proceed is minimal (~1 s) resulting in minimal blank signals.

3.1.2. Manifold selection

To simplify the FI system, in view of preparing portable instrumentation, a two-line version was developed by omitting the buffer line, directly injecting the sample in the reagent stream. To achieve efficient mixing, coils of 50, 100, 150 and 200 cm were tried. Calibration data for gallic acid in the range of 1–100 μM were obtained for these coils at flow rates of 6.3, 4.9, 3.2 and 1.6 mL min^{-1} in a 4×4 experimental design. This optimization study shows that the use of a two-line manifold is inadequate: injection of concentrated standards (50–100 μM), results in double peaks or peaks with shoulders. Moreover, peaks resulting from injection of dilute solutions are positive due to incomplete mixing with the carrier stream. Due to the above mentioned shortcomings we selected the three-line manifold set up, where sample is injected in a buffer stream.

3.1.3. Reagent–manifold–flow rate selection

Although trolox has been used for the expression of total antioxidant capacity values, gallic acid has been chosen as a model antioxidant for the reagent, manifold and flow rate optimization as it is a natural olive oil antioxidant compound.

Table 1 summarizes the results acquired during optimization of reagents loading and their optimal combination is 2.0 mM luminol, 2.0 mM *p*-iodophenol, 0.64 IU mL^{-1} HRP and 1.0 mM H_2O_2. Light emitted through the bioluminometric reaction depends on the HRP activity. Although sensitivity increases along enzyme activity, the middle concentration of 0.64 IU mL^{-1} is preferred as a compromise between sensitivity and low detection limit. Sensitivity increases by decreasing *p*-iodophenol concentration. Between the two low *p*-iodophenol concentrations (0.50, 2.0 mM) that show identical sensitivity, the 2.0 mM was chosen as the sample blank values were lower. Although sensitivity increases along hydrogen peroxide concentration, we selected 1.0 mM to protect the PMT tube detector from excessive light levels resulting from high H_2O_2 concentrations.

Table 2 summarizes results acquired during manifold and flow rate optimization. A univariate procedure using coil length 50 cm, sample loop volume 17 μL and flow rate 6.3 mL min^{-1} was applied. Best sensitivities are obtained for 50 cm and 100 cm mixing coils. We choose the 50 cm coil for higher sample throughput (180 samples/h vs 90

Table 1
Effect of reagent concentrations on the gallic acid calibration curve

Reagent	*C*	Slope ± SD, counts s^{-1} M^{-1}($\times 10^9$)	Intercept ± SD, counts s^{-1}($\times 10^6$)	DL[a], 10^{-6} M	*r*
HRP	0.13[b]	1.4 ± 0.1	0.05 ± 0.01	21	0.99
	0.64[b]	22 ± 1	0.45 ± 0.06	8.1	0.998
	3.2[b]	32 ± 2	1.2 ± 0.2	19	0.994
p-Iodophenol	0.50[c]	23 ± 2	0.17 ± 0.08	10	0.993
	2.0[c]	22 ± 2	0.15 ± 0.09	12	0.991
	5.0[c]	5 ± 1	0.15 ± 0.04	24	0.96
H_2O_2	0.50[c]	8.5 ± 0.6	0.11 ± 0.03	11	0.992
	1.0[c]	23 ± 2	0.23 ± 0.09	12	0.992
	2.0[c]	57 ± 3	0.7 ± 0.1	5.3	0.994
	3.0[c]	(7 ± 1) × 10	1.7 ± 0.4	17	0.94
	5.0[c]	140 ± 8	1.8 ± 0.4	8.6	0.995

Sample loop: 17 μL; coil: 50 cm; and flow rate: 6.3 mL min^{-1}.
Linear range of calibration curve: 1–100 μM.
[a] Detection limit, DL, was calculated as three times the standard deviation of the intercept divided by the calibration curve slope.
[b] IU mL^{-1}.
[c] mM.

Table 2
Effect of manifold parameters on the gallic acid calibration curve

Parameter		Slope ± SD, counts s^{-1} M^{-1}($\times 10^9$)	Intercept ± SD, counts s^{-1}($\times 10^6$)	DL[a], 10^{-6} M	r
Coil length (cm)	50	23 ± 1	0.40 ± 0.07	9.1	0.991
	100	25 ± 1	0.20 ± 0.08	9.6	0.98
	150	19 ± 1	0.36 ± 0.07	11	0.98
	200	17 ± 1	0.19 ± 0.06	11	0.992
Sample loop volume (μL)	17	23 ± 2	0.2 ± 0.1	13	0.993
	70	27 ± 4	0.3 ± 0.2	22	0.97
	100	27 ± 4	0.3 ± 0.2	22	0.97
Flow rate (mL min^{-1})	6.3	21 ± 1	0.21 ± 0.04	5.7	0.992
	4.9	7.5 ± 0.4	0.19 ± 0.01	6.0	0.992
	3.2	3.6 ± 0.3	0.40 ± 0.01	8.3	0.98
	1.6	0.39 ± 0.06	0.300 ± 0.002	15	0.93

2.0 mM luminol, 2.0 mM *p*-iodophenol, 0.64 IU mL^{-1} HRP, 1.0 mM H_2O_2.
Linear range was 1–100 μM.
[a] Detection limit was calculated as three times the standard deviation of the intercept divided by the calibration curve slope.

samples/h). Among sample loop volumes tested the 70 and 100 μL, have identical effect on the gallic acid curve. Although the 17 μL sample loop results in 20% approximately lower sensitivity, we choose this as lower detection limits and higher sample analysis rate have been achieved. Among flow rates tested, the 1.6, 3.2, 4.9 and 6.3 mL min^{-1} resulted in blank peak heights of 2.97, 0.36, 0.17 and 0.12×10^6 counts s^{-1}, respectively. The 6.3 mL min^{-1} flow rate is rather high for typical FI system but it was chosen on the basis of minimal blank value. This is due to less time allowed for methanol oxidation by hydrogen peroxide.

3.2. Method Evaluation

Peaks shown in Fig. 2 depict the excellent reproducibility and the minimal carry over of the proposed method. The calibration curve for the optimized method is: Peak height (counts $s^{-1} \times 10^6$) $= (23 \pm 2) \times 10^3\, C_M + (0.2 \pm 0.1)$, $r = 0.99$. Precision was evaluated by multiple injections of gallic acid standards of 1.0×10^{-6} and 1.0×10^{-4} M. Relative standard deviations were 2.8% and 1.1% ($n = 4$) respectively. Base line noise measured for 2 h was lower than 5.3% RSD. The sample throughput achieved with this fully automated method is 180 per hour.

Antioxidant efficiency of four common antioxidant compounds, mentioned in Table 3, was determined by the developed FI method. It is known that antioxidant 'power' is strongly dependent on structural factors such as number and position of hydroxyl or methoxyl groups (Mannimo et al., 1999) and is promoted by the delocalization of unpaired electrons and the formation of intramolecular hydrogen bonds. Antioxidant capacity could be related to calibration curve slopes and is in the order gallic acid > trolox > protocatechuic acid > tyrosol. Tyrosol is by

Table 3
Calibration data using different antioxidant compounds (peak height vs C, $n = 4$)

Antioxidant	Slope ± SD, counts s^{-1} M^{-1}($\times 10^{10}$)	r
Gallic acid[a]	3.96 ± 0.30	0.997
Trolox[a]	2.57 ± 0.17	0.991
Protocatechuic acid[a]	1.63 ± 0.32	0.993
Tyrosol[b]	$(1232.8 \pm 8.5) \times 10^{-5}$	0.999

Linear range: [a]1–100 μM; [b]1–100 mM.

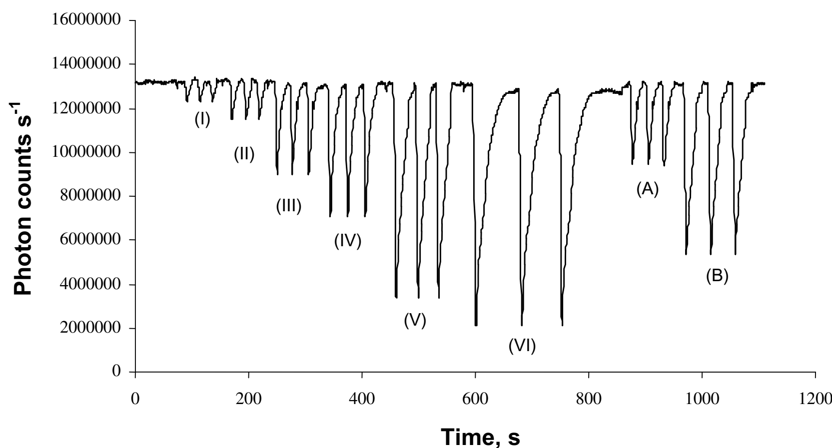

Fig. 2. Typical flow injection responses obtained by triplicate injections of blank, gallic acid standard solutions and two olive oil samples. Gallic acid concentration: (I) 0.0, (II) 1.0×10^{-6}, (III) 5.0×10^{-6}, (IV) 1.0×10^{-5}, (V) 5.0×10^{-5}, and (VI) 1.0×10^{-4} M; (A) olive oil from Arkadia; (B), olive oil from Lakonia.

Table 4
Total antioxidant capacity of different variety-origin olive oils determined through the FI-bioluminometric method

Sample no.	Origin	Variety	TAC ± SD ($n = 3$) mM gallic acid kg^{-1} olive oil	Sample no.	Origin	Variety	TAC ± SD ($n = 3$) mM gallic acid kg^{-1} olive oil
1	Chania	Koroneiki	22 ± 4	26	Lakonia	Athinolia	40 ± 4
2			51 ± 6	27			102 ± 6
3			25 ± 5	28	Zakinthos	Koroneiki	36 ± 2
4			99 ± 5	29			6.2 ± 0.3
5			72 ± 5	30			93 ± 5
6			88 ± 7	31			83 ± 1
7			4.5 ± 0.5	32			51 ± 3
8			29 ± 4	33			42 ± 4
9	Messinia		15 ± 1	34			43 ± 3
10			39 ± 3	35	Euvoia	Megaron	41 ± 4
11			21 ± 3	36	Pieria		59 ± 5
12			9.3 ± 0.7	37		Kolindrou	94 ± 4
13			36 ± 3	38	Chalkidiki	Chalkidikis	48 ± 4
14			58 ± 4	39			58 ± 5
15			14 ± 2	40		Prassinolia	20 ± 3
16			1.1 ± 0.1	41			17 ± 2
17			52 ± 3	42	Arkadia	Athinolia	14 ± 1
18		Mavrolia	88 ± 7	43	Iraklio	Koroneiki	81 ± 7
19			4.0 ± 0.3	44			88 ± 6
20	Lakonia	Mixed	96 ± 5	45			43 ± 3
21		Koroneiki	24 ± 4	46			79 ± 5
22		Athinolia	100 ± 8	47			(15 ± 1) × 10
23			91 ± 5	48	Lesbos	Kolovi	63 ± 3
24			20 ± 2	49			50 ± 2
25			53 ± 3	50		Adramatini	110 ± 8

far the least potent antioxidant. Ranking activities are in agreement with those presented in other studies (Mannimo et al., 1999; Papadopoulos et al., 2003).

3.3. Determination of olive oil extracts antioxidant capacity

The samples contained in our olive oil sample depository were analyzed in triplicate by the proposed method. Antioxidant activities were in the range of 1.1–150 mM gallic acid kg^{-1} olive oil, as shown in Table 4. This wide range of values probably reflects the variability of phenolic concentrations among olive oil samples. The proposed method was tested against the ABTS and DPPH assays not showing a clear correlation. The reasons are as follows: Antioxidant activity, estimated by the FI method, is based on different chemistry that is the reaction of antioxidant compounds with hydrogen peroxide in comparison to the reaction with stable organic free radicals used in these assays. Furthermore, the time allowed for reaction (1 s) is extremely smaller than the 1 h used in ABTS and DPPH methodologies. Beyond HRP enzyme, metal ions could also act as catalyst in the luminol oxidation by hydrogen peroxide. However, metal ions in olive oil matrix do not pass in the extract, as they are extracted only with acidic solutions (De Leonardis, Macciola, & De Felice, 2000).

4. Conclusions

In this paper an alternative assay for olive oil antioxidant capacity is presented. This bioluminometric antioxidant capacity assay is fully automated using a low cost laboratory-made FI analyzer that could be the prototype for developing a portable instrument for field evaluation of olive oil quality. In comparison to the FI methods developed for olive oil TAC assessment (Battino et al., 2004; Mannimo et al., 1999), the proposed method that is based on different detection principle, provides higher sampling rate and precision. Interference from the extraction solvent, being a result of the slow reaction of methanol with hydrogen peroxide, is kinetically masked by allowing just 1 s for reaction in the flow injection manifold measuring bioluminescence signals right after mixing. Although it would be interesting to correlate antioxidant capacities with concentrations of individual phenolics, the scope of this work is the development of an alternative antioxidant capacity assay that complements rather than replaces the existing ones for olive oil. The proposed method could be applied to any agricultural product containing antioxidant compounds reactive towards hydrogen peroxide. These antioxidants could be extracted using the different solvents or solvent mixtures tried in this study.

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