Talanta 130 (2014) 233–240

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

# Evaluation of total reducing power of edible oils

Dionysios C. Christodouleas [a,b], Charalambos Fotakis [a], Kyriakos Papadopoulos [b], Antony C. Calokerinos [a,*]

[a] Laboratory of Analytical Chemistry, Department of Chemistry, School of Sciences, National and Kapodistrian University of Athens, Panepistiomiopolis, 15771 Athens, Greece

[b] Division of Physical Chemistry, IAMPPNM, NCSR Demokritos, Agia Paraskevi Attikis, 15310 Athens, Greece

ARTICLE INFO

*Article history:*
Received 12 February 2014
Received in revised form
24 June 2014
Accepted 26 June 2014
Available online 4 July 2014

*Keywords:*
Edible oils
CUPRAC
Reducing power
Antioxidants
Fe-Phenanthroline

ABSTRACT

The lipophilicity of untreated edible oils narrows the application of most published methods for the determination of antioxidant activity to hydrophilic extracts of oils.

This research addresses the issue of the estimation of the total antioxidant properties of untreated edible oils by modifying two widely applied analytical methods, the Fe-Phenanthroline and the CUPRAC assays, to be used in untreated oils. The modifications pertain to the selection of mixture of solvents (ethanol–butanol in 3:1 v/v ratio), and the optimization of the reaction conditions (reagents concentration and reaction time).

The developed methods were applied to a number of hydrophilic and lipophilic standard compounds and different types of commercial edible oils, as well as their corresponding aqueous or organic extracts. This implementation elucidated the differences in the antioxidant content of edible oils. All the results were compared to those of the DPPH and Folin–Ciocalteu methods and the analytical figures of merit for the methods have been estimated.

Lastly, it was concluded that the modified CUPRAC assay has higher sensitivity compared to the Fe-Phenanthroline assay.



## 1. Introduction

The antioxidant activity (A.A) of natural products constitutes an important index for their nutritional value, their shelf life and their authenticity. A plethora of methodologies have been developed for the estimation of A.A of natural products mainly based on the evaluation of the scavenging activity of samples against radical species [1]. The DPPH assay [2], the ABTS assay [3], the ORAC assay [4], the luminol [5] and the lucigenin [6] chemiluminescence assays pertain to this category. In in-vivo systems, the antioxidants, in order to terminate the on-going oxidation reactions, are oxidized and act as reducing agents. Therefore, the reducing power of the test sample provides valuable information on its potential to act as an antioxidant agent. Methodologies based on the evaluation of samples' reducing activity, such as the Folin–Ciocalteu assay [7], the ferric reducing power assay using phenanthroline (Fe-Phen) [8] and tripyridyltriazine (FRAP) [9], the cerium reducing power assay (CERAC) [10], the silver reducing power assay [11] and the CUPRAC assay [12] have been used for the evaluation of the antioxidant properties of natural products.

Among the above mentioned assays, CUPRAC assay has attracted the interest of researchers during the last years due to its simple analytical procedure, the lack of need for expensive apparatus and the short reaction times [13]. The principle of the CUPRAC assay is based on the spectrophotometric monitoring of the reduction of Cu(II)–Cu(I) in a neocuproine (Nc) complex due to the presence of the antioxidants in the reaction mixture. The redox potential of $Cu(Nc)_2^{2+}/Cu(Nc)_2^{+}$ is 0.6 V, much higher than 0.17 V which is the redox potential of $Cu^{2+}/Cu^{1+}$, therefore the reduction of cupric ions is feasible by the antioxidant compounds. The analytical signal is the increase on the absorbance value of 450 nm due to the formation of Cu(I)–neocuproine complex after 10–30 min of the mixing of the reagents [12]. The reaction that takes place is shown in the following equation:

$$n[Cu(Nc)_2]^{2+} + Ar(OH)_n \rightarrow n[Cu(Nc)_2]^{+} + Ar(=O)_n + nH^{+} \quad (1)$$

CUPRAC assay has been used for the determination of a plethora of antioxidant compounds and natural products [13,14]. The reaction solvent that has been used until now is mainly a mixture of ethanol-water. The use of dichloromethane and an acetone–water mixture as reaction solvent has been also reported [15–17].

* Corresponding author. Tel.: +30 210 7274316; fax: +30 210 7274750.
*E-mail address:* calokerinos@chem.uoa.gr (A.C. Calokerinos).

http://dx.doi.org/10.1016/j.talanta.2014.06.058
0039-9140/

The Fe-Phen assay is also a simple and easily utilized method that estimates the reducing capacity of a sample. It is based on the spectrophotometric monitoring, at 510 nm, of the reduction of Fe (III)–Fe(II) in a phenanthroline (phen) complex due to the presence of the antioxidants in the reaction mixture. The time required for the reduction is 1–10 min [8]. The reaction that takes place is shown in the following equation:

$$n[\mathrm{Fe(phen)_3}]^{3+} + \mathrm{Ar(OH)}_n \rightarrow n[\mathrm{Fe(phen)_3}]^{2+} + \mathrm{Ar(=O)}_n + n\mathrm{H}^+ \quad (2)$$

Edible oils are the main source of fat in contemporary diet and a source of important micronutrients, such as vitamins, carotenes and polyphenols [18]. In particular, the oil type, the extraction method, the place of origin and the cultivation methods influence the concentration of the nutrient constituents of oil. Therefore, according to these factors, the diet is supplemented by different antioxidants. Extra virgin olive oil is a great source of hydrophilic antioxidants, such as phenolic compounds [19,20], and seed oils a great source of lipophilic antioxidants such as tocopherols [21]. Due to the lipophilicity of edible oils, the majority of published methods for the determination of their antioxidant activity are usually restricted to extracts of oils [6,21–25]. Nevertheless, any treatment of oil prior to analysis, such as extraction, changes the chemical composition of the tested sample and lead to erroneous results. Hence, direct application of analytical methods to oil without any pretreatment except dilution would be preferable [26]. A limited number of methods have been developed for the determination of A.A of edible oils without prior treatment [27–34]. The luminescent methods that have been developed for the determination of total antioxidant activity of edible oils have been extensively reviewed recently [35]. To the best of our knowledge CUPRAC and Fe-Phen assays have been only applied to assess the antioxidant profile of oils' hydrophilic extracts [36,37] and not of untreated oils.

The purpose of the present work is to expand the use of CUPRAC and Fe-Phen assays to hydrophobic natural products, such as edible oils. The developed CUPRAC and Fe-Phen modified assays could be used for the evaluation of the total reducing power of untreated edible oils. The reaction solvent which has been chosen is a mixture of ethanol–butanol in 3:1 v/v ratio due to the high solubility of the necessary reagents, the hydrophilic and lipophilic test compounds, and the untreated oils in this medium. The developed methods were applied to various edible oils and the obtained results were compared to those of the DPPH and Folin–Ciocalteu methods.

## 2. Materials and methods

### 2.1. Apparatus

Absorption measurements were performed on a JASCO V-500 spectrophotometer.

### 2.2. Reagents and solutions

All chemicals were of analytical purity and were used without further purification. 1-Butanol, hexane, methanol and ethanol were purchased from Panreac. Oleuropein was purchased from Extrasynthese. Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), caffeic acid, ascorbic acid, catechin α-tocopherol, gallic acid, chlorogenic acid, pyrocatechol, cupric acetate, ferric chloride, ammonium acetate were purchased from Sigma-Aldrich. Neocuproine and phenanthroline were obtained from Across Organics.

All commercial edible oils, (extra virgin olive oils, refined olive oils, sunflower oil, soybean oils, sesame oil and corn oil) were purchased from local supermarkets.

Aqueous stock solutions of cupric ions (0.100 M), ferric ions (0.100 M), and ammonium acetate ions (1.00 M) were prepared and stored at 4 °C. Antioxidant standard stock solutions were prepared by accurately weighing and dissolving with ethanol. Working solutions of phenanthroline and neocuproine were prepared daily by accurately weighing and dissolving with ethanol.

### 2.3. Sample preparation

The total reducing power (TRP) of edible oils was measured by using solutions of 4.0% v/v of edible oils in 1-butanol. For the estimation of the contribution of the corresponding hydrophilic or lipophilic extracts to TRP of edible oils, solutions of hydrophilic and lipophilic extracts, 4.0% v/v of the extracts in 1-butanol, were prepared as following:

10.00 g of the oil sample was diluted in 10 mL hexane, and the hydrophilic part was extracted three times with 20 mL methanol/water (60/40 v/v) by centrifugation for 10 min at 3000 cpm. The hydrophilic and the hydrophobic extracts were collected and the solvents were removed in a rotary evaporator under vacuum at temperatures of about 50 °C. To the solid residue of the hydrophilic extract, 12.74 mL of 1-butanol was added and the mixture was stirred vigorously. Then, 0.40 mL of this solution was diluted to 5.00 mL with 1-butanol in order to obtain a 4.0% v/v hydrophilic extract solution of the oil. 0.40 mL of the hydrophobic extract was also diluted to 5.00 mL with 1-butanol in order to obtain a 4.0% v/v hydrophobic extract solution of the oil.

### 2.4. Spectrophotometric determination of total reducing power using a modified CUPRAC assay

1.00 mL of $7.00 \times 10^{-3}$ M neocuproine solution in ethanol, 1.00 mL of $2.00 \times 10^{-3}$ M cupric ions solution in ethanol, 1.00 mL of $1.00 \times 10^{-2}$ M ammonium acetate solution in ethanol and 1.00 mL of tested solution diluted in 1-butanol were mixed and the absorbance value of the reaction mixture at 450 nm was measured after 30 min.

### 2.5. Spectrophotometric determination of total reducing power using a modified Fe-Phen assay

1.00 mL of $6.00 \times 10^{-3}$ M phenathroline solution in ethanol, 1.00 mL of $1.00 \times 10^{-3}$ M ferric ions solution in ethanol, 1.00 mL of $1.00 \times 10^{-2}$ M ammonium acetate in ethanol and 1.00 mL of tested solution diluted in 1-butanol were mixed and the absorbance value of the reaction mixture at 510 nm was measured after 5 min.

### 2.6. Spectrophotometric determination of total reducing power using the original CUPRAC and Fe-Phen assays

For the CUPRAC assay, 1.00 mL of $7.00 \times 10^{-3}$ M neocuproine solution in ethanol, 1.00 mL of $2.00 \times 10^{-3}$ M aqueous Cu(II) solution, 1.00 mL of $1.00 \times 10^{-2}$ M aqueous ammonium acetate and 1.00 mL of tested solution diluted in ethanol were mixed and the absorbance value of the reaction mixture at 450 nm was measured after 30 min.

For the Fe-Phen assay, 1.00 mL of $6.00 \times 10^{-3}$ M phenathroline solution in ethanol, 1.00 mL of $1.00 \times 10^{-3}$ M aqueous Fe(III) solution, 1.00 mL of $1.00 \times 10^{-2}$ M aqueous ammonium acetate and 1.00 mL of tested solution diluted in ethanol were mixed and the absorbance value of the reaction mixture at 510 nm was measured after 5 min.

### 2.7. Spectrophotometric determination of scavenging activity against DPPH• of untreated edible oils

A modified DPPH method [38] was used for the evaluation of A. A of oils. 4.0 mL of $8.75 \times 10^{-5}$ M DPPH in ethyl acetate was mixed with 1.0 mL of solvent (blank) or hydrophilic extract solution in ethyl acetate and after 1 h, the absorbances $A_o$ or $A$, respectively, were measured at 515 nm. A.A(%) is calculated by the following equation:

$$\text{A.A}(\%) = \frac{A_o - A}{A_o} \times 100 \quad (3)$$

Three measurements were performed for each test solution. The results were expressed as mg gallic acid equivalents per liter of edible oil (mg GAE/L).

### 2.8. Spectrophotometric determination of total phenols in hydrophilic extract of edible oils

A modified Folin–Ciocalteu method was used for the determination of total phenols content [39]. An aliquot of 10.00 mL of the hydrophilic extract of edible oils was diluted with 10.00 mL distilled water, mixed with 2.00 mL of Folin–Ciocalteu reagent (2 N) and stirred. 5.00 mL of sodium carbonate solution (7.5 % w/v) was then added and the mixture was stirred vigorously. The mixture was diluted to 50.00 mL with distilled water, incubated to 45 °C into a water bath for 15 min and the absorbance at 750 nm was recorded. The results were expressed as mg gallic acid equivalents per liter of edible oil (mg GAE/L).

### 2.9. Statistical analysis

All measurements were obtained in five replicates and values were averaged and reported along with the standard deviation (S.D.). The Kolmogorov–Smirnov tests were applied to the results of the 4 spectrophotometric assays to examine normality. A non-normal distribution was detected and the Spearman correlation was applied to extract the correlation coefficients. These calculations were performed with the SPSS (IBM SPSS Statistics. version 19.0. Chicago. IL. USA) statistical software for Windows.

## 3. Results and discussion

### 3.1. Method development

The already proposed CUPRAC and Fe-Phen assays [8,13–17,36,37] cannot be applied to untreated edible oils due to the presence of water in the reaction mixture and consequent formation of turbid solutions. The selection of the proper solvent or mixture of solvents and the optimization of the reaction conditions (reagents concentration and reaction time) were delineated at first.

#### 3.1.1. Solvent selection

A number of solvents (2-propanol, 1-propanol, chloroform, dichloromethane, acetone, hexane, ethanol and 1-butanol) and mixtures of them have been tested as possible reaction solvent(s). Hexane, 1-propanol and 2-propanol reduce cupric ions and therefore they could not be used as assay solvents. Chloroform, acetone and dichloromethane mix with the reagents of the assay but the first is toxic solvent, whereas the latter presents drawbacks due to volatility, which alters the solution concentration. Ethanol and 1-butanol cannot dilute the reaction mixtures when used alone but various mixtures of them can be used successfully. For both the CUPRAC and Fe-Phen methods, a 3:1 v/v ethanol–butanol mixture was chosen as the reaction solvent. This reaction mixture was selected due to the very good solubility of all the reagents required as well as the hydrophilic and lipophilic antioxidants and the untreated oils.

#### 3.1.2. Reagent optimization

The concentration of the used reagents has been also optimized in order to achieve the maximum absorbance values when $5.00 \times 10^{-5}$ M gallic acid solution was used as the test sample.

*CUPRAC*: In this assay, the concentration of the Cu(II) solution was optimized in the range of 0.60–6.00 mM in the presence of $8.00 \times 10^{-3}$ M neocuproine and $1.00 \times 10^{-2}$ M ammonium acetate. The results indicated that the optimum concentration of Cu(II) ions was $2.00 \times 10^{-3}$ M (Fig. 1A). The concentration of neocuproine solution was optimized in the concentration range of 1.00–10.00 mM in the presence of $2.00 \times 10^{-3}$ M Cu(II) and $1.00 \times 10^{-2}$ M ammonium acetate solutions. The results indicated that the optimum concentration of neocuproine was $7.00 \times 10^{-3}$ M (Fig. 1B). The concentration of ammonium acetate was optimized in the concentration range of 0.10–100 mM in the presence of $2.00 \times 10^{-3}$ M Cu(II) and $7.00 \times 10^{-3}$ M neocuproine solutions. The results probed to the independence of the absorption of the reaction mixture from the concentration of the ammonium acetate solution, thus the concentration of $1.00 \times 10^{-2}$ M was used. Therefore the optimum concentrations of Cu(II), neocuproine, and ammonium acetate solutions were found to be equal to $2.00 \times 10^{-3}$ M, $7.00 \times 10^{-3}$ M and $1.00 \times 10^{-2}$ M, respectively and were used for all further studies. Lastly, the reaction time was optimized. A reaction mixture was prepared with the above reagent concentrations and the absorbance was recorded every 5 min. The absorbance values reached a plateau after 30 min.

*Fe-Phen*: In this assay, the concentration of Fe(III) solution was optimized in the concentration range of 0.1–10 mM in the presence of $8.00 \times 10^{-3}$ M phenanthroline and $1.00 \times 10^{-2}$ M ammonium acetate. The results indicated that the optimum concentration of Fe(III) solution was $1.00 \times 10^{-3}$ M (Fig. 1C). The concentration of phenanthroline solutions was optimized in the concentration range of 0.1–10 mM in the presence of $1.00 \times 10^{-3}$ M Fe(III) and $1.00 \times 10^{-2}$ M ammonium acetate. The results indicated that the optimum concentration of phenantroline solutions was $6.00 \times 10^{-3}$ M (Fig. 1D). It was also observed that the absorbance values were independent from the ammonium acetate concentration in the range of 0.10–100 mM in the presence of $1.00 \times 10^{-3}$ M Fe(III) and $6.00 \times 10^{-3}$ M phenanthroline solutions, thus the concentration of $1.00 \times 10^{-2}$ M was used. Therefore the optimum concentrations of Fe(III), phenantroline, and ammonium acetate solutions were found to be equal to $1.00 \times 10^{-3}$ M, $6.00 \times 10^{-3}$ M and $1.00 \times 10^{-2}$ M, respectively and were used for all further studies. Finally, it was concluded that the absorbance values reached a plateau after 5 min.

### 3.2. Application to hydrophilic and hydrophobic compounds

The modified CUPRAC and Fe-Phen assays were applied to evaluate the reducing power of nine standard compounds, both hydrophilic and hydrophobic. The response curves displaying the absorbance vs the concentration of each antioxidant have been prepared, using five standard solutions each one measured five times (Tables 1 and 2). A close inspection on these measurements probed to the higher sensitivity of the CUPRAC assay over the Fe-Phen assay since the detection limits are lower in all cases.

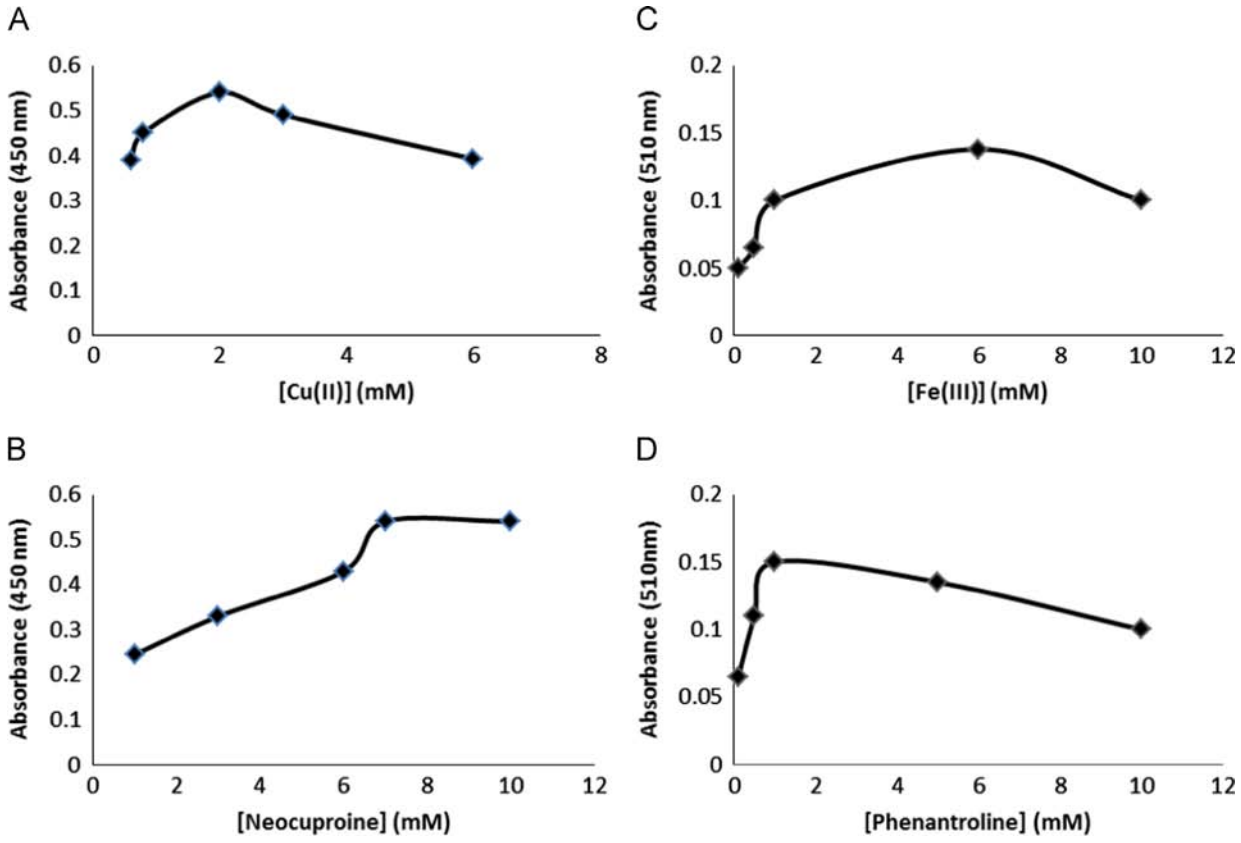


**Fig. 1.** The effect of (1A) concentration of Cu(II) solution in the presence of $8.00 \times 10^{-3}$ M neocuproine, (1B) concentration of neocuproine in the presence of $2.00 \times 10^{-3}$ M Cu(II), (1C) concentration of Fe(III) solution in the presence of $8.00 \times 10^{-3}$ M phenanthroline and (1D) concentration of phenanthroline in the presence of $1.00 \times 10^{-3}$ M Fe (III) (all solutions in $1.00 \times 10^{-2}$ M ammonium acetate) on the absorbance from $5.00 \times 10^{-5}$ M gallic acid by the CUPRAC (1A, 1B) and Fe-Phen (1C, 1D) methods.

**Table 1**
Regression lines (Abs$=A+B \times C$) of 9 antioxidants by the modified CUPRAC method.

| Ranking | Compound | $Abs_{0.5}$ (μM), ±S.D. ($N=5$) | GERP | Linear range (μM) | $A \times 10^{-3}(\pm S_A)$ | $B \times 10^{3}(\pm S_B)$ | LoQ (μM) | LoD (μM) | $R$ ($n=8$) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | (±)-Catechine | 45.4 ± 1.9 | 0.91 | 10–200 | −4.08 (±14.7) | 11.1 (±1.6) | 13.24 | 4.37 | 0.9995 |
| 2 | Gallic acid | 49.9 ± 2.1 | 1 | 8–200 | 0.6 (±12) | 10.0 (±1.4) | 12.00 | 3.96 | 0.9995 |
| 3 | Caffeic acid | 61.5 ± 2.6 | 1.23 | 8–200 | 18 (±15) | 7.84 (±1.7) | 19.13 | 6.31 | 0.998 |
| 4 | Oleurepein | 71.2 ± 3.0 | 1.43 | 10–200 | 4.2 (±7.4) | 6.96 (±0.87) | 10.63 | 3.51 | 0.9995 |
| 5 | Chlorogenic acid | 79.0 ± 3.3 | 1.58 | 10–200 | −25.6 (±6.3) | 6.65 (±0.59) | 9.47 | 3.13 | 0.9998 |
| 6 | Pyrocatechol | 89.1 ± 3.7 | 1.78 | 20–200 | −136 (±36) | 7.14 (±0.30) | 50.42 | 16.64 | 0.998 |
| 7 | Ascorbic acid | 114.2 ± 4.8 | 2.29 | 10–200 | −3.8 (±5.3) | 4.41 (±0.59) | 12.02 | 3.97 | 0.9995 |
| 8 | Trolox | 136.7 ± 5.7 | 2.74 | 15–300 | −25.0 (±7.7) | 3.84 (±0.52) | 20.05 | 6.62 | 0.9996 |
| 9 | α-Tocopherol | 172.6 ± 7.2 | 3.46 | 10–200 | −17.9 (±5.5) | 3.00 (±0.56) | 18.33 | 6.05 | 0.9993 |

The concentration of the antioxidants that lead to absorbance values of 0.5 ($Abs_{0.5}$) and 0.05 ($Abs_{0.05}$) units have been estimated for the CUPRAC and Fe-Phen assays respectively (Tables 1 and 2). These values have been used for the estimation of the Gallic acid Equivalent Reducing Power (GERP) value of each compound by using Eqs. (4) and (5).

$$GERP_{CUPRAC} = \frac{Abs_{0.5}(antioxidant)}{Abs_{0.5}(Gallic\ acid)}, \quad (4)$$

$$GERP_{Fe-Phen} = \frac{Abs_{0.05}(Antioxidant)}{Abs_{0.05}(Gallic\ acid)}, \quad (5)$$

It is preferable to facilitate GERP values, rather than $Abs_{0.5}$ and $Abs_{0.05}$ values due to the strong dependence of the latter on reaction parameters and conditions used. Therefore the antioxidant activity was expressed relative to a reference compound measured under the same conditions, namely gallic acid.

The GERP values have been used for ranking of antioxidants according to their reducing power (Tables 1 and 2). Specifically, for the CUPRAC assay the trend observed was the following: (±)-Catechin > Gallic acid > Caffeic acid > Oleuropein > Chlorogenic acid > Pyrocatechol > Ascorbic acid > Trolox > α-tocopherol. On the other hand, for the Fe-Phen assay another trend was elicited: Gallic acid > Ascorbic acid > Caffeic acid > Oleuropein > Chlorogenic acid > (±)-Catechin > Pyrocatechol > α-tocopherol > Trolox.

Gallic acid is among the compounds with the highest reducing properties in both assays, while α-tocopherol and its synthetic analog trolox the weakest. Furthermore, caffeic acid, oleuropein and chlorogenic acid shared the same ranking position in both assays. Interestingly, ascorbic acid and (±)-catechin exhibited a

**Table 2**
Regression lines (Abs$=A+B\times\log C$) of 9 antioxidants by the modified Fe-Phen method.

| Ranking | Compound | $Abs_{0.05}$ (μM), ± S.D. ($N=5$) | GERP | Linear range (μM) | $A(\pm S_A)$ | $B(\pm S_B)$ | LoQ (μM) | LoD (μM) | $R$ ($n=5$) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Gallic acid | 10.51 ± 0.52 | 1 | 10–200 | 527 (± 24) | 95.8 (± 3.6) | 20.33 | 6.71 | 0.998 |
| 2 | Ascorbic acid | 18.34 ± 0.91 | 1.75 | 10–200 | 1045 (± 93) | 210 (± 22) | 87.68 | 28.94 | 0.98 |
| 3 | Caffeic acid | 29.6 ± 1.4 | 2.82 | 10–200 | 382 (± 20) | 73.3 (± 4.5) | 24.09 | 7.95 | 0.995 |
| 4 | Oleurepein | 30.2 ± 1.5 | 2.87 | 20–200 | 823 (± 55) | 171 (± 12) | 34.91 | 11.52 | 0.995 |
| 5 | Chlorogenic acid | 49.5 ± 2.4 | 4.71 | 20–200 | 394 (± 25) | 79.9 (± 4.6) | 32.66 | 10.78 | 0.9991 |
| 6 | (±)-Catechine | 62.4 ± 3.1 | 5.94 | 20–200 | 584 (± 52) | 127 (± 12) | 68.02 | 22.45 | 0.99 |
| 7 | Pyrocatechol | 558 ± 27 | 53.1 | 70–800 | 197.7 (± 20) | 45.4 (± 1.5) | 86.15 | 28.43 | 0.998 |
| 8 | α-Tocopherol | 836 ± 41 | 79.5 | 50–200 | 121.1 (± 9.2) | 23.1 (± 2.2) | 62.48 | 20.62 | 0.993 |
| 9 | Trolox | 1900 ± 95 | 180 | 10–200 | 83.2 (± 3.5) | 12.20 (± 0.54) | 26.80 | 8.85 | 0.995 |

**Table 3**
Total reducing power of edible oils (TRP) as well as hydrophilic ($RP_{hydro}$) and lipophilic extracts ($RP_{lipo}$) solutions of 4.0% v/v by using the CUPRAC method.

| Edible oil | RP G.A.E ($\times 10^{-5}$ M) (± S.D., $n=5$) | | | | % Difference |
|---|---|---|---|---|---|
| | TRP | $RP_{lipo}$ | $RP_{hydro}$ | Sum of $RP_{lipo}$ and $RP_{hydro}$ extracts | |
| EVOO 1 | 18.49 (± 0.22) | 18.35 (± 0.33) | 0.81 (± 0.02) | 19.16 | −3.6 |
| VOO | 19.96 (± 0.50) | 19.44 (± 0.47) | 0.52(± 0.01) | 19.45 | 2.6 |
| EVOO 2 | 19.99 (± 0.64) | 18.97 (± 0.55) | 0.55 (± 0.01) | 19.53 | 2.3 |
| EVOO 3 | 17.19 (± 0.48) | 19.11 (± 0.32) | 0.13 (± 0.01) | 19.25 | −11.9 |
| Corn oil | 13.76 (± 0.33) | 16.49 (± 0.30) | 0.24 (± 0.02) | 16.74 | −21.3 |
| Sun oil | 23.49 (± 0.85) | 27.49 (± 0.71) | 0.83 (± 0.05) | 28.33 | −20.6 |
| Soya oil | 20.03 (± 0.74) | 19.86 (± 0.64) | 1.58 (± 0.02) | 21.44 | −7 |
| Sesame oil | 17.84 (± 0.50) | 17.74 (± 0.99) | 0.64 (± 0.01) | 18.39 | −3 |

**Table 4**
Total reducing power of edible oils (TRP) as well as hydrophilic ($RP_{hydro}$) and lipophilic extracts ($RP_{lipo}$) solutions of 4.0% v/v by using the Fe-Phen method.

| Edible oil | RP G.A.E ($\times 10^{-6}$ M) (± S.D., $n=5$) | | | | % Difference |
|---|---|---|---|---|---|
| | TRP | $RP_{lipo}$ | $RP_{hydro}$ | Sum of $RP_{lipo}$ and $RP_{hydro}$ extracts | |
| EVOO 1 | 18.42 (± 0.44) | 8.60 (± 0.30) | 4.98 (± 0.24) | 13.58 | −26.3 |
| VOO | 8.44 (± 0.22) | 6.60 (± 0.20) | 4.74 (± 0.22) | 11.34 | 34.4 |
| EVOO 2 | 9.96 (± 0.30) | 8.05 (± 0.22) | 4.36 (± 0.20) | 12.41 | 24.6 |
| EVOO 3 | 12.72 (± 0.24) | 7.92 (± 0.20) | 5.58 (± 0.22) | 13.5 | 6.1 |
| Corn oil | 32.04 (± 0.88) | 26.36 (± 0.96) | – | 26.36 | −17.7 |
| Sun oil | 34.24 (± 0.98) | 25.64 (± 0.48) | – | 25.64 | −25.1 |
| Soya oil | 29.54 (± 0.97) | 20.48 (± 0.56) | – | 20.48 | −30.7 |
| Sesame oil | 10.26 (± 0.45) | 12.98 (± 0.46) | – | 12.98 | 26.5 |

converse relation among the two assays. The differences observed in antioxidant activity of some compounds by the Fe-Phen and CUPRAC assays can be attributed to the different measurement principles.

### 3.3. Application to edible oils

The preparation of solutions of oils which exhibit absorbance values within the linear range of the method requires the appropriate oil dilution. Therefore, different oil dilutions were examined corroborating that the final solution for measurement should be 4.0% v/v. Gallic acid was chosen as the antioxidant compound to express reducing power for edible oils using Eq. (6) for the CUPRAC assay and Eq. (7) for the Fe-Phen assay. The corresponding Gallic Acid Equivalent (GAE) values have been estimated.

$$\text{Abs(units)} = 0.6\times 10^{-3} + 10\times 10^{3}\times C_{\text{gallic acid}}, \quad (6)$$

$$\text{Abs(units)} = 527.2 + 95.8\times \log C_{\text{gallic acid}}, \quad (7)$$

The total reducing power (TRP), as well as the contribution of the hydrophilic ($RP_{hydro}$) and the lipophilic ($RP_{lipo}$) extracts to the total reducing power (TRP) of oils estimated by the two assays are shown in Tables 3 and 4. It is obvious that the estimation of antioxidant properties of edible oils based only on their hydrophilic extracts underestimate the true antioxidant properties of edible oils. Interestingly, it was also revealed that only the CUPRAC assay managed to estimate the reducing power of both extracts in all the types of edible oil, while the Fe-Phen assay failed to estimate the weak reducing power of hydrophilic extracts of seed oils.

In the case of olive oils, the results from the CUPRAC assay prove that the contribution of their lipophilic part (16.49–27.49 G.A.E ($\times 10^{-5}$ M)) to the total RP is significantly higher than that of their hydrophilic (0.13–1.58 G.A.E ($\times 10^{-5}$ M)). The Fe-Phen assay reveals a very different trend and provides lower values. In fact, it probes to the equal contribution of the lipophilic and hydrophilic part of olive to its total RP as they are approximately of the same order of magnitude. It has been also reported that the A.A of aqueous extracts of olive oils is mainly attributed to phenolic compounds derived from benzoic acid or cinnamic acids as well as to their corresponding alcohols, flavonoids, secoiroids, and lignans, while the A.A of their organic extracts is mainly

attributable to lipophilic compounds such as tocopherols, carotenoids (β-carotene, lutein), chlorophylls, and pheophytins. In addition, the organic extracts of olive oil also contain phenolic compounds (approximately 5% of total phenols), such as hydroxyltyrosylmalate and tyrosyloleates which are not extractable in the aqueous phase due to their lipophilic character [40].

The remarkably high antioxidant activity of lipophilic extracts of seed oils is possibly attributable to the high content on lipophilic compounds, such as tocopherols and polyunsaturated fatty acids [21].

### 3.4. Method validation

The quality characteristics of each method (linearity, precision, accuracy, limit of detection and limit of quantification) were also investigated. The limits of detection (LoD) and quantitation (LoQ) were calculated. The former corresponds to $\mathrm{LoD} = 3.3 \times s_a/b$ and the latter is calculated by the following equation $\mathrm{LoQ} = 10 \times s_a/b$, where $s_a$ corresponds to the standard error of the intercept and $b$ the slope of the calibration lines.

*Linearity* was established by measuring the absorbance of at least five solutions of each antioxidant compound at known concentrations. Each measurement was repeated five times and the mean value was used for calculation of the regression lines.

*Precision* was evaluated by estimating the repeatability (intra-day precision) and reproducibility (inter-day precision) of the method. The former was investigated by estimating the standard deviation of the five measurements of each antioxidant compound. The latter was calculated by measuring the reducing power of six edible oils and three gallic acid solutions at three different days.

*Accuracy* was estimated by using recovery assays, due to the lack of a reference material or a standard method for the estimation of antioxidant properties of tested samples. The recovery experiments were carried out by measuring the TRP of 4.0% v/v oil solutions before and after spiking with gallic acid. Recovery was calculated by using Eq. (8) and results are shown in Tables 3 and 4.

$$\text{Recovery}(\%) = \frac{\text{TRP of spiked samples}}{\text{expected TRP}} \times 100 \quad (8)$$

**Table 5**
Reducing power of edible oils 4.0% v/v before and after spiking with $1.00 \times 10^{-4}$ M gallic acid by using the CUPRAC method.

| Edible oil | RP G.A.E ($\times 10^{-5}$ M) ($\pm$S.D., $n=5$) (%RSD) | | | |
|---|---|---|---|---|
| | Total oil | Spiked oil | Expected value | Recovery (%) |
| EVOO 1 | 18.49 ($\pm$0.22) (−1.2) | 27.56 ($\pm$0.50) (−1.8) | 28.49 | 97 |
| VOO | 19.96 ($\pm$0.50) (−2.5) | 29.65 ($\pm$0.71) (−2.4) | 29.96 | 99 |
| EVOO 2 | 19.99 ($\pm$0.64) (−3.2) | 31.20 ($\pm$0.90) (−2.9) | 29.99 | 104 |
| EVOO 3 | 17.19 ($\pm$0.48) (−2.8) | 26.45 ($\pm$0.45) (−1.7) | 27.19 | 97 |
| Corn oil | 13.76 ($\pm$0.33) (−2.4) | 25.78 ($\pm$0.46) (−1.8) | 23.76 | 108 |
| Sun oil | 23.49 ($\pm$0.85) (−3.6) | 32.56 ($\pm$0.85) (−2.6) | 33.49 | 97 |
| Soya oil | 20.03 ($\pm$0.74) (−3.7) | 31.20 ($\pm$0.99) (−3.2) | 30.03 | 104 |
| Sesame oil | 17.84 ($\pm$0.50) (−2.8) | 29.87 ($\pm$1.70) (−5.7) | 27.84 | 107 |

**Table 6**
Reducing power of edible oils 4.0% v/v before and after spiking with $1.00 \times 10^{-5}$ M gallic acid by using the Fe-Phen method.

| Edible oil | RP G.A.E ($\times 10^{-6}$ M) ($\pm$S.D., $n=5$) (%RSD) | | | |
|---|---|---|---|---|
| | Total oil | Spiked oil | Expected value | Recovery (%) |
| EVOO 1 | 18.42 ($\pm$0.44) (2.4) | 27.74 ($\pm$0.49) (1.7) | 28.42 | 98 |
| VOO | 8.44 ($\pm$0.22) (2.6) | 17.92 ($\pm$0.47) (2.6) | 18.44 | 97 |
| EVOO 2 | 9.94 ($\pm$0.30) (3.0 | 18.52 ($\pm$0.27) (1.5) | 19.94 | 93 |
| EVOO 3 | 12.72 ($\pm$0.24) (1.9) | 24.84 ($\pm$0.61) (2.5) | 22.72 | 109 |
| Corn oil | 32.04 ($\pm$0.88) (2.7) | 46.88 ($\pm$0.91) (1.9) | 42.04 | 112 |
| Sun oil | 34.24 ($\pm$0.99) (2.9) | 39.04 ($\pm$0.93) (2.4) | 44.24 | 88 |
| Soya oil | 29.74 ($\pm$0.89) (3.0) | 42.82 ($\pm$0.98) (2.3) | 39.74 | 108 |
| Sesame oil | 10.3 ($\pm$0.46) (4.5) | 25.68 ($\pm$0.93) (3.62) | 20.3 | 127 |

The validation results are presented below:

*CUPRAC*: All nine antioxidant compounds examined exhibit antioxidant properties and can be measured at the sub-millimolar concentration with correlation coefficients within the range 0.998–0.9998 (Table 1). Specifically, gallic acid, which was selected as the reference compound to express the antioxidant activity of edible oils, its correlation coefficient was 0.9995 and is deemed satisfactory. For all compounds, the linear range was at least one order of magnitude and the detection and quantification limits for each compound are shown in Table 1. The intra-day precision was 2.5% and the inter-day precision 4.2%, these values were deemed satisfactory. The mean recovery (Table 5) was estimated to be equal to $98 \pm 5.2$ % ($n=8$) which was satisfactory as verified by Student test at a confidence level of 95% ($t_{\mathrm{theor}}=2.365 > t_{\mathrm{exp}}=0.43$) postulating that the method does not exhibit systematic error.
*Fe-Phen*: All nine compounds which exhibit antioxidant activity can be measured at the sub-millimolar concentration with correlation coefficients within the range 0.98–0.999. Particularly gallic acid, the selected reference compound used to express the antioxidant activity of edible oils, displayed a satisfactory correlation coefficient of $>0.99$. For all tested compounds, the linear range was at least one order of magnitude (Table 2) and the detection and quantification limits for each compound are shown in Table 2. The intra-day precision was 3.3% and the inter-day precision 6.7%, these values were deemed satisfactory. The mean recovery (Table 6) was estimated to be equal to $102.7 \pm 4.8$ % ($n=8$) which was satisfactory as verified by Student test at a confidence level of 95% ($t_{\mathrm{theor}}=2.365 > t_{\mathrm{exp}}=0.1$) postulating that the method does not exhibit systematic error.

### 3.5. Comparison with the original CUPRAC and Fe-Phen assays

The original experimental protocols of the CUPRAC and Fe-Phen methods cannot be employed in oils, so the comparison of the results obtained from the original and the modified methodologies was performed by testing standard compounds. Nine antioxidant compounds have been tested using the original CUPRAC and Fe-Phen assays and employing the same reagent concentrations between the original and the modified assays to

**Table 7**
Regression lines (Abs=$A+B\times C$) of 9 antioxidants by the original CUPRAC method.

| Ranking | Compound | $Abs_{0.5}$ (μM), ±S.D. ($N=5$) | GERP | Linear range (μM) | $A\times10^{-3}(\pm S_A)$ | $B\times10^{3}(\pm S_B)$ | LoQ (μM) | LoD (μM) | $R$ ($n=8$) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Gallic acid | 46.69 | 1.00 | 7–100 | −57 (±12) | 11.94 (±0.23) | 10.44 | 3.44 | 0.999 |
| 2 | (±)-Catechin | 48.20 | 1.03 | 5–100 | −8.4 (±10) | 10.55 (±0.21) | 9.96 | 3.29 | 0.998 |
| 3 | Chlorogenic acid | 75.77 | 1.62 | 20–200 | 95 (±24) | 5.33 (±0.23) | 46.05 | 15.19 | 0.995 |
| 4 | Pyrocatechol | 84.35 | 1.81 | 20–200 | −14 (±40) | 6.10 (±0.37) | 65.67 | 21.67 | 0.99 |
| 5 | Oleurepein | 85.73 | 1.84 | 5–100 | 25 (±11) | 5.53 (±0.23) | 21.44 | 7.08 | 0.991 |
| 6 | Caffeic acid | 92.24 | 1.98 | 5–200 | 25.4 (±4.6) | 5.14 (±0.05) | 9.08 | 2.99 | 0.9993 |
| 7 | Trolox | 102.99 | 2.21 | 5–200 | 15.6 (±8.0) | 4.70 (±0.09) | 17.05 | 5.63 | 0.998 |
| 8 | Ascorbic acid | 123.85 | 2.65 | 5–200 | −8.2 (±8.6) | 4.10 (±0.10) | 20.88 | 6.89 | 0.996 |
| 9 | α-Tocopherol | 162.71 | 3.49 | 5–200 | −15.6 (±9.8) | 3.17 (±0.11) | 30.94 | 10.21 | 0.994 |

**Table 8**
Regression lines (Abs=$A+B\times C$) of 9 antioxidants by the original Fe-Phen method.

| Ranking | Compound | $Abs_{0.05}$ (μM), ±S.D. ($N=5$) | GERP | Linear range (μM) | $A\times10^{-3}(\pm S_A)$ | $B\times10^{3}(\pm S_B)$ | LoQ (μM) | LoD (μM) | $R$ ($n=8$) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Oleurepein | 6.26 | 0.03 | 5–100 | 15 (±11) | 5.62 (±0.23) | 20.44 | 6.75 | 0.992 |
| 2 | Caffeic acid | 8.51 | 0.04 | 10–200 | 36.5 (±5.9) | 1.58 (±0.06) | 37.55 | 12.39 | 0.994 |
| 3 | α-Tocopherol | 30.41 | 0.16 | 5–200 | 17.3 (±3.3) | 1.07 (±0.04) | 31.03 | 10.24 | 0.992 |
| 4 | Ascorbic acid | 44.22 | 0.23 | 7–200 | 0.03 (±0.96) | 0.45 (±0.01) | 21.33 | 7.04 | 0.997 |
| 5 | (±)-Catechine | 100.91 | 0.52 | 7–200 | 18.9 (±1.1) | 0.31 (±0.01) | 38.25 | 12.62 | 0.991 |
| 6 | Chlorogenic acid | 171.86 | 0.88 | 20–200 | 8.6 (±12.2) | 0.24 (±0.01) | 506.28 | 167.07 | 0.992 |
| 7 | Gallic acid | 195.10 | 1.00 | 7–200 | 6.23 (±0.98) | 0.22 (±0.01) | 43.75 | 14.44 | 0.992 |
| 8 | Pyrocatechol | 209.34 | 1.07 | 20–200 | 11.00 (±0.83) | 0.19 (±0.01) | 43.53 | 14.36 | 0.995 |
| 9 | Trolox | N.D.[a] | N.D.[a] | 20–200 | 116.1 (±6.9) | 0.17 (±0.01) | 39.71 | 13.10 | 0.996 |

[a] The value of the intercept was higher than 0.05 absorbance units.

**Table 9**
Phenolic content of the hydrophilic extract of edible oils measured by the Folin–Ciocalteu (F–C) assay and the antioxidant capacity of untreated edible oils measured by the DPPH assay.

| Edible oils | DPPH | F–C |
|---|---|---|
| | mM GAE/L ±S.D. ($n=3$) | |
| EVOO 1 | 1.091 ± 0.034 | 0.778 ± 0.008 |
| VOO | 0.891 ± 0.011 | 0.535 ± 0.006 |
| EVOO 2 | 0.712 ± 0.017 | 0.569 ± 0.006 |
| EVOO 3 | 1.112 ± 0.011 | 0.531 ± 0.009 |
| Corn oil | 3.522 ± 0.066 | N.D[a] |
| Sun oil | 1.288 ± 0.032 | N.D[a] |
| Soya oil | 2.743 ± 0.011 | N.D[a] |
| Sesame oil | 1.486 ± 0.023 | N.D[a] |

[a] Not detectable.

**Table 10**
Linear correlation coefficients among the results of DPPH, CUPRAC, Fe-Phen and Folin–Ciocalteu (F–C) methods.

| Pearson, Correlation coefficient | $F–C_{hydro}$ | DPPH | CUPRAC | | Fe-Phen | |
|---|---|---|---|---|---|---|
| | | | TRP | $RP_{hydro}$ | TRP | $RP_{hydro}$ |
| $F–C_{hydro}$ | 1 | | | | | |
| DPPH | 0.37 | 1 | | | | |
| CUPRAC | | | | | | |
| TRP | −0.12 | −0.54 | 1 | | | |
| $RP_{hydro}$ | −0.78 | 0.18 | 0.52 | 1 | | |
| Fe-Phen | | | | | | |
| TRP | 0.89** | 0.69* | 0.07 | 0.37 | 1 | |
| $RP_{hydro}$ | −0.03 | 0.9* | −0.95 | −0.6 | −0.39 | 1 |

* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.

get comparable results. The response curves displaying the absorbance vs the concentration of each antioxidant have been prepared, using five standard solutions each one measured five times (Tables 7 and 8). The concentration of each antioxidant which lead to absorbance values of 0.5 ($Abs_{0.5}$) and 0.05 ($Abs_{0.05}$) units, for the CUPRAC and the Fe-Phen assay respectively, and the GERP values have been also estimated (Tables 7 and 8).

By comparing the results of Table 1 to those of Table 7, it was concluded that the original and the modified CUPRAC assays give comparable results in terms of relative reactivity of the antioxidants and method sensitivity, estimated form the limit of detections. The only significant difference between the results of the two assays was the relative reactivity of caffeic acid since in the modified CUPRAC assay, it was ranked third in the relative ranking and in the original method sixth. On the other hand, the original Fe-Phen assay gave significantly different results compared to the modified Fe-Phen assay (Tables 2 and 8). The original assay proved to be more sensitive than the modified assay and the ranking of antioxidants according to their reducing power was completely different.

The differences between the results of the modified and the original methods can be attributed to the solvent effect which influences the transfer of electrons from the antioxidants to the metal ions of the complexes. The fact that the results of the original and the modified CUPRAC assays are similar highlights the robustness of the assay to different solvents.

### 3.6. Comparison with other methods

The total reducing power estimated by CUPRAC and Fe-Phen methods was compared with the total phenol content of oils estimated by the Folin–Ciocalteu (F–C) method and the scavenging activity of oil against DPPH• (Table 9). The F–C method in comparison to the two modified assays, provided much higher GAE values probably due to the much higher reactivity of Folin–Ciocalteu reagent against all kinds of phenol compounds contained in oils. Moreover, the F–C and the Fe-Phen methods could not evaluate the antioxidant activity of hydrophilic extracts in seed oils, probably due to the very small amount of phenolic antioxidants in these extracts. On the other hand, the modified CUPRAC

and DPPH methods accomplished to assess the antioxidant activity of hydrophilic extracts in seed oils.

Interestingly all the implemented methods attested to the higher antioxidant activity of the corn, sun and soya oils compared to the olive oils.

To a step further, correlation analysis between the results obtained by all four methods has been performed and the results are shown in Table 10. In fact, the results of the TRP in the Fe-Phen assay were significantly correlated to those of the DPPH and the F–C assay, while the results of the $RP_{hydro}$ in the Fe-Phen assay was significantly correlated only to those obtained by the DPPH assay. On the other hand, the values of reducing power estimated by CUPRAC were not significantly correlated to any of the three assays, indicating different responses of this method to the antioxidant compounds present in oil. The differences depicted in the correlation analysis can be attributed to the different analytical principles of each method. This finding necessitates the implementation of CUPRAC in order to fully assess the antioxidant profile of a food item.

## 4. Conclusions

In this study, two analytical assays, based on CUPRAC and Fe-Phen methods, have been proposed for the estimation of the total reducing power of edible oils as well as of their corresponding aqueous or organic extracts. The original experimental protocol of the CUPRAC and Fe-Phen methods cannot be employed to assess the total antioxidant activity of edible oils. Therefore tweaking these methods in order to alleviate this obstacle has expanded their analytical implementation and improved their scalability.

The application of the two methods to different kinds of oils revealed the differences in the antioxidant content for each type of edible oil. The results showed that total reducing power of olive oils is lower than those of seed oils. The contribution of A.A of their corresponding aqueous and organic extracts to the total A.A of the whole oil is different for olive oils compared to seed oils. The aqueous extracts of seed oils show negligible A.A, whereas their lipophilic extracts contribute more than 90% to the total reducing of whole oil. Thus, the antioxidant activity of seed oils should be measured in untreated oil samples and not in aqueous extracts, otherwise the true antioxidant content of seed oils is underestimated.

The comparison of the results of the two methods to the results obtained by using two other methods, F–C and DPPH methods, revealed that the results of different methods are not completely comparable and interchangeable in characterizing the antioxidant capacity of edible oil. Then it can be corroborated that the differences in mechanism of each assay render necessary the implementation of more than one spectrophotometric assay in order to evaluate its antioxidant activity.

The total antioxidant properties of edible oils are of considerable interest because they can be used as a quality index for the shelf-life and the stability of edible oils. Various authors have demonstrated a positive linear relationship between the concentration of antioxidant compounds in edible oil and the oil stability [41,42]. The enhanced stability and self-life of edible oils with increasing antioxidant content is attributed to the ability of antioxidants to inhibit lipid oxidation, the main process that leads to the quality deterioration, degradation and off flavor formation in edible oils. The modified CUPRAC and Fe-Phen methods are simple, quick, accurate, precise, do not require expensive instrumentation or experienced personnel and could be easily be incorporated in kits for in-field measurements. Therefore, they have all the quality factors required for the routine analysis of commercial edible oils in industrial or in-field settings.

## Acknowledgments

This research has been co-financed by the European Union (European Social Fund – ESF) and Greek National Funds through the Operational Program “Education and Lifelong Learning” of the National Strategic Reference Framework (NSRF) – Research Funding Program: THALES. Investing in knowledge society through the European Social Fund.

## References

[1] A. Karadag, B. Ozcelik, S. Saner, Food Anal. Methods 2 (2009) 41–60.
[2] M.N. Xanthopoulou, E. Fragopoulou, K. Kalathara, T. Nomikos, H.C. Karantonis, S. Antonopoulou, Food Chem. 120 (2010) 665–672.
[3] J. Lachman, M. Sulc, M. Schilla, Food Chem. 103 (2007) 802–807.
[4] H. Li, X. Wang, Y. Li, P. Li, H. Wang, Food Chem. 112 (2009) 454–460.
[5] D.L. Giokas, A.G. Vlessidis, N.P. Evmiridis, Anal. Chim. Acta 589 (2007) 59.
[6] K. Papadopoulos, T. Triantis, E. Yannakopoulou, A. Nikokavoura, D. Dimotikali, Anal. Chim. Acta 494 (2003) 41.
[7] S. McDonald, P.D. Prenzler, M. Antolovich, K. Robards, Food Chem. 73 (2001) 73.
[8] K.I. Berker, K. Guclu, I. Tor, R. Apak, Talanta 72 (2007) 1157–1165.
[9] V. Katalinic, M. Milos, D. Modun, I. Music, M. Boban, Food Chem. 86 (2004) 593–600.
[10] D. Ozyurt, B. Demirata, R. Apak, J. Fluoresc. 21 (2011) 2069–2076.
[11] M. Özyürek, N. Güngör, S. Baki, K. Güçlü, R. Apak, Anal. Chem. 84 (2012) 8052–8059.
[12] R. Apak, K. Guclu, B. Demirata, M. Ozyurek, S.E. Celik, B. Bektasoglu, K.I. Berker, D. Ozyurt, Molecules 12 (2007) 1496–1547.
[13] M. Özyürek, K. Güçlü, R. Apak, TrAC-Trends Anal. Chem. 30 (2011) 652–664.
[14] M. Özyürek, K. Güçlü, E. Tütem, K.S. Başkan, E. Erçağ, S.E. Çelik, S. Baki, L. Yıldız, Ş. Karaman, R. Apak, Anal. Methods 3 (2011) 2439–2453.
[15] R. Apak, K. Güçlü, M. Özyürek, S.E. Karademir, J. Agric. Food Chem. 52 (2004) 7970–7981.
[16] S.E. Çelik, M. Özyürek, K. Güçlü, R. Apak, Talanta 81 (2010) 1300–1309.
[17] M. Özyürek, B. Bektaşoğlu, K. Güçlü, N. Güngör, R. Apak, Anal. Chim. Acta 630 (2008) 28–39.
[18] M.J. Navas, A.M. Jime´nez, J. Am. Oil Chem. Soc. 84 (2007) 405–411.
[19] M. Servili, R. Selvaggini, S. Esposto, A. Taticchi, G. Montedoro, G. Morozzi, J. Chromatogr. A 1054 (2004) 113–127.
[20] A. Carrasco-Pancorbo1, L. Cerretani, A. Bendini, A. Segura-Carretero, T. Gallina-Toschi, A. Fernandez-Gutierrez, J. Sep. Sci. 28 (2005) 837–858.
[21] C.I.G. Tuberoso, A. Kowalczyk, E. Sarritzu, P. Cabras, Food Chem. 103 (2007) 1494–1501.
[22] S. Gorinstein, O. Martin-Belloso, E. Katrich, A. Lojek, M. Cız, N. Gligelmo-Miguel, R. Haruenkit, Y.S. Park, S.T. Jung, S. Trakhtenberg, J. Nutr. Biochem. 14 (2003) 154–159.
[23] F. Saura-Calixto, I. Goni, Food Chem. 94 (2006) 442–447.
[24] A. Szydłowska-Czerniak, C. Dianoczki, K. Recseg, G. Karlovits, E. Szłyk, Talanta 76 (2008) 899–905.
[25] K.S. Minioti, C.A. Georgiou, Food Chem. 109 (2008) 455–461.
[26] V. Gökmen, A. Serpen, V. Fogliano, Trends Food Sci. Technol. 20 (2009) 278–2886-7 20 (2009) 278–288.
[27] S. Mannino, S. Buratti, M.S. Cosio, N. Pellegrini, Analyst 124 (1999) 1115–1118.
[28] J.C. Espin, C. Soler-Rivas, H.J. Wichers, J. Agric. Food Chem. 48 (2000) 648–656.
[29] N. Pellegrini, F. Visioli, S. Buratti, F. Brighenti, J. Agric. Food Chem. 49 (2001) 2532–2538.
[30] J.L. Quiles, M.C. Ramirez-Tortoza, J.A. Gomez, J.R. Huertas, J. Mataix, Food Chem. 76 (2002) 461–468.
[31] K.X. Hay, V.Y. Waisundara, M. Timmins, B. Ou, K. Pappalardo, N. Mchale, D. Huang, J. Agric. Food Chem. 54 (2006) 5299–5305.
[32] D. Christodouleas, C. Fotakis, K. Papadopoulos, E. Yannakopoulou, A.C. Calokerinos, Anal. Chim. Acta 652 (2009) 295–302.
[33] A. Nikokavoura, D. Christodouleas, E. Yannakopoulou, K. Papadopoulos, A.C. Calokerinos, Talanta 84 (2011) 874–880.
[34] D. Christodouleas, K. Papadopoulos, A.C. Calokerinos, Food Anal. Methods 4 (2011) 475–484.
[35] D. Christodouleas, C. Fotakis, K. Papadopoulos, D. Dimotikali, A.C. Calokerinos, Anal. Lett. 45 (2012) 625–641.
[36] S.E. Çelik, M. Özyürek, K. Güçlü, R. Apak, Anal. Biochem. 423 (2012) 36–38.
[37] A. Szydłowska-Czerniak, C. Dianoczki, K. Recseg, G. Karlovits, E. Szłyk, Talanta 76 (2008) 899–905.
[38] J.C. Espın, C. Soler-Rivas, H.J. Wichers, J. Agric. Food Chem. 48 (2000) 648–656.
[39] S. McDonald, P.D. Prenzler, M. Antolovich, K. Robarts, Food Chem. 73 (2001) 73–84.
[40] G. Montedoro, M. Servilli, M. Baldioli, E. Miniati, J. Agric. Food Chem. 40 (1992) 1571–1576.
[41] T. Gutfinger, J. Am. Oil Chem. Soc. 58 (1981) 966–968.
[42] M. Baldioli, M. Servili, G. Perretti, G.F. Monedoro, J. Am. Oil Chem. Soc. 73 (1996) 1589–1593.