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Evaluation of total reducing power of edible oils

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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].

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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 7.00×10^{-3} M neocuproine solution in ethanol, 1.00 mL of 2.00×10^{-3} M cupric ions solution in ethanol, 1.00 mL of 1.00×10^{-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 6.00×10^{-3} M phenathroline solution in ethanol, 1.00 mL of 1.00×10^{-3} M ferric ions solution in ethanol, 1.00 mL of 1.00×10^{-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 7.00×10^{-3} M neocuproine solution in ethanol, 1.00 mL of 2.00×10^{-3} M aqueous Cu(II) solution, 1.00 mL of 1.00×10^{-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 6.00×10^{-3} M phenathroline solution in ethanol, 1.00 mL of 1.00×10^{-3} M aqueous Fe(III) solution, 1.00 mL of 1.00×10^{-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.