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Multicommuted flow injection method for fast photometric determination of phenolic compounds in commercial virgin olive oil samples

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ABSTRACT

A multicommuted flow injection method has been developed for the determination of phenolic species in virgin olive oil samples. The method is based on the inhibitory effect of antioxidants on a stable and colored radical cation formation from the colorless compound *N,N*-dimethyl-*p*-phenylenediamine ($DMPD^{\bullet+}$) in acidic medium in the presence of Fe(III) as oxidant. The signal inhibition by phenolic species and other antioxidants is proportional to their concentration in the olive oil sample. Absorbance was recorded at 515 nm by means of a modular fiber optic spectrometer. Oleuropein was used as the standard for phenols determination and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) was the reference standard used for total antioxidant content calculation. Linear response was observed within the range of 250–1000 mg/kg oleuropein, which was in accordance with phenolic contents observed in commercial extra virgin olive oil in the present study. Fast and low-volume liquid–liquid extraction of the samples using 60% MeOH was made previous to their insertion in the flow multicommuted system. The five three-way solenoid valves used for multicommuted liquid handling were controlled by a homemade electronic interface and Java-written software. The proposed approach was applied to different commercial extra virgin olive oil samples and the results were consistent with those obtained by the Folin Ciocalteu (FC) method. Total time for the sample preparation and the analysis required in the present approach can be drastically reduced: the throughput of the present analysis is 8 samples/h in contrast to 1 sample/h of the conventional FC method. The present method is easy to implement in routine analysis and can be regarded as a feasible alternative to FC method.

1. Introduction

Extra virgin olive oil (EVOO) is a key component of the traditional Mediterranean diet to which health benefits are attributed [1–4] due to its high level of both (i) phenolic compounds with a powerful antioxidant activity (mainly phenols and tocopherols) and (ii) unsaturated fatty acids with a high monounsaturated: polyunsaturated ratio. This EVOO unique feature composition is the responsible of its higher resistance to oxidation compared to the rest of vegetal oils.

Phenols play a key role on the antioxidant activity of virgin olive oil (VOO) as they are regarded as the molecules with the highest potential to block free radicals acting as primary antioxidants by donating a radical hydrogen to alkylperoxyl radicals formed during the initiation step of lipid oxidation so forming a stable radical. On the other hand, they have been recognized as potential nutraceutic compounds for food and pharmaceutical industries [5].

Due to their antioxidant properties, considerable research efforts are being devoted to the phenolic compounds and a plethora of methodologies have been proposed to test antioxidant activity of foods in general [6,7] including VOO [8,9] making use of different principles such as ability to scavenge free radicals or measurement of total reducing capacity being 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), oxygen radical antioxidant capacity (ORAC), ferric reducing ability of plasma (FRAP) and cupric reducing antioxidant capacity (CUPRAC) assays the most commonly used [10–12]. The non-specific colorimetric assay based on the Folin–Ciocalteu reagent is probably the most used procedure for the determination of phenolic species in VOO [13] and other samples. It

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is a classical method for total phenolic quantitation, based on their reducing ability. Two important drawbacks of this procedure are: (a) its low specificity (the color reaction can occur with any oxidizable phenolic hydroxyl group, even with non-phenolic compounds [14]); and (b) the labor and time consuming reaction (1–2 h). Recently, methodological approaches to improve the specificity of the FC assay for total phenolic content determinations have been reviewed [15]. For extra virgin olive oil (EVOO) samples, some correlations have been established between FC assay and other antioxidant assays, as DPPH, ABTS and ORAC, being the best correlation obtained between FC and ABTS [11]. All these assays are also time consuming either for the reaction or for the radical formation.

In spite of the efforts devoted to the development methodologies for the determination of phenols/antioxidants contents in VOO, as far as we know, there is no simple test method able to reflect the antioxidant profile of a VOO sample, probably due to the complexity of the antioxidant processes in food samples. Therefore, the development of new methods for evaluating phenols/antioxidants of VOO samples that may circumvent some of the drawbacks of currently available methods is of great interest.

In this sense, some attempts to automate FC method for the determination of phenolic compounds in olive oil have been performed [16–18] based on: (i) the combination of robotics and Flow Injection Analysis (FIA) to carry out the unattended FC assay on the samples [19], which takes 30 min. per analysis; (ii) on-line liquid–liquid extraction using iterative flow reversal approach coupled to a flow-through spectrophotometric sensor with increased sampling rates [20]; and (iii) ultrasound-assisted liquid–liquid extraction without phase separation [21]. Although these methods include the automatic sample extraction step of phenols, they are either very expensive [20] or tedious and not robust enough [21] to be applied to routine analysis. Nevertheless, scarce attention has been paid to the automation of alternative assays based on color development reactions for VOO samples. N,N-dimethyl-p-phenylenedimine (DMPD) has been proposed for measurement of antioxidant activity of wines based on the absorbance inhibition of its radial cation $DMPD^{\bullet+}$ [22], which were comparable to those by other available procedures such as ABTS assay. Additionally, antioxidant activity obtained by DMPD assay was well correlated with the phenolic content calculated by FC method. However, DPMD assay has been scarcely investigated for antioxidant activity and total phenolic content measurements in olive oil [23].

Multicommuted flow analysis (MCFA) is based on the use of a set of computer-controlled 3-way solenoid valves to design flow manifolds, allowing to increase flow system versatility with low both sample and reagent consumptions (reagents are used in the minimum amounts and just in the necessary moment of the analytical procedure) and low waste volumes. MCFA approach can be considered as an evolution of flow injection analysis (FIA) towards Green Analytical Chemistry [24,25]. Examples of applications of MCFA can be found in [24]. Recently, a chemiluminescence procedure based on a multicommuted flow system has been described for phenolic compounds in several food samples, including olive oil [26]. The procedure is simple and attractive for some types of food matrices, however the sample treatment for olive oil matrix is complex and time consuming and involves relatively high solvent volume consumption.

The aim of this article is to investigate the use of $DMPD^{\bullet+}$ for the estimation of total phenolic content in EVOO samples by means of a multicommuted flow injection analysis (MCFA) procedure. MCFA is proposed as a fast and automatic alternative to the classical FC procedure, easy to implement in routine laboratories.

2. Experimental

2.1. Reagents and solutions

All experiments were performed with analytical-reagent grade reagents. A Milli-Q-Plus ultra-pure water system from Millipore (Milford, MA, USA) was used throughout the study to obtain highly pure water for the preparation of standard solutions, samples and reagents. Sodium acetate (AcNa), methanol HPLC grade, *N,N*-dimethyl-*p*-phenylenediamine (DMPD), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), gallic acid, tyrosol and Folin–Ciocalteu reagent were purchased from Sigma-Aldrich (Madrid, Spain). Oleuropein was from Extrasynthese (Genay Cedex, France), Fe(III) chloride, hydrochloric acid and sodium carbonate were obtained from Panreac (Barcelona, Spain).

2.1.1. Carrier solution

Working solution of acetate buffer 0.1 mol L^{-1} was prepared by dissolving sodium acetate in Milli-Q water and adjusting the pH to 5.25 using diluted HCl solution.

2.1.2. Fe(III) solutions:

Stock solution of 50 mmol L^{-1} from $FeCl_3$ was prepared with Milli-Q water. Daily working solution of 1 mmol L^{-1} was prepared by appropriate dilution with milliQ water and acidified up to pH 1 with HCl.

2.1.3. DMPD solution

Working solution of 4 mmol L^{-1} from DMPD was daily freshly prepared with Milli-Q water. This solution is stable during 4 h at room temperature (see Section 3.1.1).

2.2. Reference method for the determination of total phenolic content of EVOO samples

The total phenolic content of EVOO samples was determined by using the Folin–Ciocalteu reagent [16,27]. First, the phenolic compounds were extracted from samples using a mixture of methanol/water. Then, the obtained extract is mixed with the Folin–Ciocalteu reagent to obtain a colored solution.

2.2.1. Extraction procedure

10 g of the sample of EVOO were weighted (with a precision of 0.1 g) and dissolved in 50 mL of hexane. The mixture is loaded into a 100-mL separatory funnel to undergo a three step liquid–liquid extraction. First, 10 mL of a solution of methanol/water (60:40 v/v) were added and the mixture was vigorously shaken for 2 min; then the hydroalcoholic phase (lower) was collected in a 50-mL volumetric flask. This extraction step was repeated two times more, being all the hydroalcoholic phases combined in the same flask. Finally, the volumetric flask is made up to the mark with distilled water.

2.2.2. Colorimetric Folin–Ciocalteu reaction

The chromogenic reaction is carried out in a 50-mL volumetric flask. First, 30 mL of distilled water were added, followed by 5 mL of the EVOO extract and 2.5 mL of Folin–Ciocalteu reagent. The mixture is shaken to homogenize. Three minutes later, 5 mL of a sodium carbonate saturated solution in water were added and the flask was made to the mark with distilled water. Then, the homogenized mixture was left to stand for 1 h at room temperature. The absorbance of the resulting blue-colored solution was finally measured at 765 nm against blank solution. The concentration of the total phenolic content was determined by a comparison with the values obtained with standard solutions of gallic acid (ranging from 1 to 10 mg/L) subjected to the same