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Response surface methodology for the optimization of phenolic compounds extraction from extra virgin olive oil with functionalized gold nanoparticles

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ABSTRACT

The optimization of phenolic compounds extraction from extra virgin olive oil (EVOO) was achieved applying a Response Surface Methodology. A simple and straightforward method for the extraction of phenolic compounds from EVOO has been optimized in the presence of functionalized gold nanoparticles (AuNPs). AuNPs were prepared in the presence of Cysteamine as stabilizing thiol (AuNPs-Cys) in order to obtain a water stable colloidal suspension. The obtained AuNPs-Cys, with mean size of about 10 nm were observed by AFM and used as extracting phase of phenolic compounds from EVOO. External parameters influencing the extraction were studied and a Central Composite Design (CCD) was applied for the optimization. The role of AuNPs-Cys quantity (4–12 mg), mixing and contact time (30–120 min), interfering (olive oil quantity) and phenolic compounds concentration (50–500 ppm) were analyzed. An equation model was obtained by applying CCD, with a high significativity ($p < 0.0001$) and low coefficient of variation value (CV = 3.14%). Optimization of extraction conditions were modulated up to 99%. This model allows to select the optimal extraction conditions to achieve the best performances and can be widely applied.

1. Introduction

Polyphenols represent one of the most important and ubiquitous groups of plant metabolite and, ranging from simple phenolic molecules to highly molecular weight compounds, their occurrence in plant foods is extremely variable [1]; they are one of the most important group of natural compounds arising from fruits, olive oil, wine and tea [2,3]. Scientific interest in food phenolic compounds has increased recently, owing to their possible beneficial implications in human health, such as their anti-microbial, anti-carcinogenic effects and their antioxidant activity, demonstrated in vivo and in vitro experiments [4]. Their possible effects against cardiovascular diseases and neurodegenerative disorders were deeply investigated [5,6] and several studies continue to be carried out to in depth investigate their health potential and to study the mechanisms of action [7,8]. Italy represented in the past years, the second European and Worldwide producer of Extra Virgin Olive Oil (EVOO), although recently its production has halved, as a result of the crisis in this sector made by the Xylella infection [9,10]. As a consequence, the importation of EVOO has exceeded the production in recent years, then the possibility of fraud has increased. Phenolic compounds are typical constituents of EVOO and could be considered like "quality molecular markers"; moreover, they also have a great interest for their organoleptic and healthy properties [11,12]. EVOO contains mainly fatty acid, and the phenolic fraction represents the 2% of total oil composition also with sterols, hydrocarbons, alcohols, pigments, tocopherols and volatile aromatic substances [3,13,14]. Phenolic compounds content in EVOO depends on the level of maturity, the growing conditions, the cultivars [15] and other several factors, for this reason the same plant could have different content in phenolic compound, both qualitatively and quantitatively [16,17]. Polyphenols in EVOO occur in different forms: tyrosol, hydroxytyrosol and their derivatives, derivatives of 4-hydroxybenzoic acid and 4-hydroxyphenylacetic, lignans, secoiridoids and flavonoids [18,19]. This variety of forms of phenolic compounds makes their extraction from matrix and their analysis extremely complex [20]. The qualitative and quantitative determination of phenolic compounds in oil samples is very important for its quality [21] and origin control [22,23]. In the last years, the determination of phenolic fraction of extra virgin olive oil has been widely studied [2,24] and a lot of developments of analytical methods are discussed in literature [25,26]. The total phenolics content can be determined by spectrophotometric method among which the most used is the Folin-Ciocalteu (FC) method.

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FC's reagent is composed by phosphomolybdic and phosphotungestic acid that reduce the phenolic compounds and change his colour. It's possible to quantify this colour changing by measuring the absorbance at a specific wavelength [27]. This and other techniques need a preliminary extraction of phenolic compounds from EVOO and for this reason a lot of studies were applied to develop and to optimize the better extraction method. Even if several methods are still being developed, such as ultrasound assisted extraction, microwave assisted extraction [5] or supercritical fluid extraction [28], the easier solid phase extraction [5] and the liquid/liquid extraction [29,30] are usually chosen. Generally, the extraction is carried out with a hydroalcoholic phase of methanol and water mixture at different percentage, then the extraction is followed by the removal of solvents and a subsequent concentration. This kind of extraction has some limits like the variation of percentage of water (to extract more of less polar compounds) or the involvement of large volumes of extraction phases [26].

In this framework, nanoparticles can be used, taking advantage of their unique surface properties and high surface/volume ratio [31,32]. Among others, gold nanoparticles (AuNPs) offer high stability, easy chemical synthesis and a wide range of surface functionalizations [33,34] also with the use of water or eco-friendly solvents and procedures [35]. AuNPs have been studied as suitable materials for applications ranging from sensors [36] to biotechnology [37,38], drug delivery [39,40], nanomedicine [41], food control [42] and optoelectronics [43]. These applications often require nanoparticles with low size (below 100 nm) and low size distribution. These features can be achieved by the versatile wet reduction of Au(III) precursors with strong reducing agents [44]. A key role in their properties can be envisaged in the stabilizing layer: in particular the chemistry of thiols has been deeply investigated and the presence of different end groups confers to the AuNPs a hydrophilic or a hydrophobic behaviour. For example, the use of thiols bearing aminic end groups has been recently proposed and hydrophilic AuNPs have been deeply investigated [45]. It is noteworthy that AuNPs are considered generally non-toxic, allowing their use for example food analysis [46] and as tools for quality control [47].

In this context, AuNPs have been used in a vast set of applications in analytical chemistry. As it has recently been reviewed [48], one important piece of work has been dedicated to the use of AuNPs for the evaluation of antioxidant activity.

In this work, hydrophilic AuNPs have been functionalized with Cysteamine (AuNPs-Cys) as stabilizing layer. A rapid and simple AuNPs-based extraction procedure has been conjugated to a new type of extraction method of phenolic compounds in a complex matrix: extra virgin olive oil (EVOO). AuNPs-Cys are chosen for the contact with olive oil in order to load phenolic compounds on the surface of NPs and then to release them in a specific volume of solvent. The study is carried out to optimize the best loading conditions that to lead at the best extraction efficacy by using less amount of NPs, solvents and time. This valuable feature is exploited to develop a new extraction method assay for phenols assessment in fatty matrices with high food significance.

2. Materials and methods

2.1. Materials

Methanol (CH_3OH), n-Hexane (C_6H_{14}), Folin-Ciocalteu reagent ($H_3[P(W_3O_{10})_4]/H_3[P(Mo_3O_{10})_4]$), Gallic acid ($C_7H_6O_5$), hydrochloric acid (HCl), sodium hydroxide (NaOH) and sodium carbonate (Na_2CO_3), standard olive oil, were purchased from Sigma Aldrich Chemical Co. Cysteamine hydrochloride ($HSCH_2CH_2NH_2{\cdot}HCl$, Cys 98%), tetrachloroauric(III) acid trihydrate ($HAuCl_4{\cdot}3H_2O$, 99.9%), sodium borohydride ($NaBH_4$, 98%), were used as received (Aldrich reagent grade). Deionized water has been degassed for 30 min with Argon, before use.

2.2. Synthesis and characterization of AuNPs-Cys

AuNPs-Cys samples were prepared according to literature report [49], adapting the procedure for the cysteamine thiol. In particular, the Au/S molar ratio has been changed from 1/4 to 1/1 and optimized for this study with the Au/S = 1/1.5 M ratio (see Supporting Information section). The synthesis is herein reported in detail for AuNPs-Cys (Au/S = 1/1.5). Starting from 200 mg of $HAuCl_4{\cdot}3H_2O$ in 10 mL of deionized water, a solution of Cys (0.0865 g, 7.6×10^{-4} mol in 10 mL of deionized water) was added. After 10 min of stirring, the suspension was bubbled under nitrogen at room temperature for 10 min; then the reducing agent $NaBH_4$ (0.1437 g, 3.86×10^{-3} mol in 10 mL of deionized water; molar ratio Au/reducing agent equal of 1/5) was added under vigorous stirring. The mixture was allowed to react for 2 h at room temperature, under magnetic stirring. The product has been purified by centrifuge washing with ultrapure water for 5 times at 5000 rpm for 10 min. The total time of purification is about 75 min. Yield: 35 ± 5% wt. The synthetic procedure was carried out 15 times.

AuNPs-Cys: UV–vis (λ_{max}, nm H_2O): 553; DLS: $<2R_H>$, nm, H_2O: 50 ± 10; FTIR (cm^{-1}, film): 3311, 2840, 1125, 923, 741, 669.

Cys thiol reference: UV–vis (λ_{max}, nm H_2O): 244; FTIR (cm^{-1}, film): 3341 (νNH), 2852, 2778 (νCH_2), 2446 (νSH), 1431, 1320 (νCN), 1200, 1060, 929, 832, 645.

2.3. Liquid-liquid extraction of phenolic compounds from EVOO

A liquid-liquid extraction (LLE) was performed in order to quantify the phenolic compounds. Sample extractions for total phenols content were prepared from 1 g of extra virgin olive oil in 2 mL of n-hexane by adding 2 mL of methanol: water (70:30) mixture. Samples were stirred for 1 min and then centrifuged at 3000 rpm for 5 min, then the hydroalcoholic fraction was collected in an amber vial. This operation was repeated twice.

2.4. AuNPs-Cys extraction of phenolic compounds from EVOO

Preliminary experiments were carried out on standard solutions; in order to mimic olive oil the standard solution was made by Gallic Acid (GA) in Ethyl Acetate (10 mg/mL) and different aliquots of this solution (in the range 15–150 μL) were mixed with n-Hexane up to a final volume of 3 mL [3]. Different quantities of AuNPs-Cys (4, 8, 12 mg) were introduced in the solution and used to extract phenolic compounds. Two methods of contact were tested: i.e. stirring or sonication, both for different mixing time (from 30 min to 2 h) [50]. In a typical procedure, 500 ppm (150 μL) of the GA solution in Ethyl acetate/Hexane, were stirred with 4 mg of AuNPs-Cys for 2 h. After 2 h the mixtures were centrifuged for 5 min at 3000 rpm in order to separate AuNPs. The surnatant was transferred in a new vial and a LLE and a total phenolic compounds determination was performed. The same procedure was carried out with a standard olive oil, mixing 1.00 g of olive oil in 2 mL of n-hexane in the presence of 4 mg of AuNPs-Cys. All the experiments were conducted in triple.

2.5. Determination of total phenolic compounds (Folin-Ciocalteu)

Total phenolics content was determined using the Folin-Ciocalteu method [51]. The method was modified for EVOO as follows: 1 mL of hydroalcoholic extract was added to 0.25 mL of FC reagent, 0.5 mL of Na_2CO_3 (7.5% w/v), in a 10 mL volumetric flask reaching the final volume with purified water. Each sample was stored for 45 min at room temperature, and the spectrophotometric analysis was performed at λ = 750 nm. Total phenolics content was expressed as milligrams GA equivalent (GAE) per Kg, the final results were obtained through a calibration curve with range from 15 to 500 μg mL^{-1} ($R^2 = 0.9925$). All the experiments were conducted in triple.