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Simple and rapid silver nanoparticles based antioxidant capacity assays: Reactivity study for phenolic compounds

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ABSTRACT

A single-step, rapid (10 min), sensitive silver nanoparticles (AgNPs) based spectrophotometric method for antioxidant capacity (AOC) assay has been developed. The assay is based on the ability of natural polyphenols to reduce Ag(I) and stabilize the produced AgNPs(0) at room temperature. Localized surface plasmon resonance (LSPR) of AgNPs at ≈420 nm is then measured. Using different conditions of pH (8.4) and temperature (45 °C) a further assay based on the production of AgNPs with selectivity for flavonols was also developed. The reactivity of the two AgNPs based assays vs. 15 polyphenols belonging to different chemical classes and 9 different samples has been studied and compared with ABTS, Folin and AuNPs based methods for AOC. The proposed assays had good reproducibility (RSD ≤ 13) and are simple, sensitive and cost effective. Moreover, used in conjunction with the classical AOC assays, can improve the information on the polyphenolic pool of food samples.

1. Introduction

Polyphenols are ubiquitous secondary metabolites present in plant foods (El Gharras, 2009). The common structural feature of all polyphenols, the presence of phenolic hydroxyl group(s), is the basis of their antioxidant activity in vitro and in vivo. In food matrices, antioxidants prevent fat rancidity and decrease the adverse effects of reactive oxygen (ROS) and nitrogen species (RNS). In general, an antioxidant reacting with a free radical, yields an electron, is oxidized, and produce a weak, non-toxic free radical that is stable and unable to propagate the reaction. The antioxidant properties of polyphenols are mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Pisoschi, Cimpeanu, & Predoi, 2015).

Since antioxidants are molecules able to slow down or prevent the oxidation of other molecules, a diet rich in antioxidants has been associated with a reduced risk of developing some pathologies; in particular, epidemiological studies and associated meta-analyses suggest prevention of development of cancer, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases (Del Rio et al., 2012; Lall, Syed, Adhami, & Khan, 2015; Pandey & Rizvi, 2009).

Polyphenols have been also demonstrated to be key modulators of signalling pathways and to influence micro-RNA expression (Lall et al., 2015; Zhang & Tsao, 2016).

Foods rich in polyphenols are widely used in dietary formulations and an increasing number of research papers have appeared in the literature on the discovery and application of natural antioxidants and their therapeutic and technological properties (El Gharras, 2009; Shahidi & Zhong, 2010).

The development of efficient procedures for the extraction, proper analysis and characterization of phenolic compounds from different sources is a challenging task due to the structural diversity of the compounds, complexity of natural sources and their interaction with other components of the matrix. Different analytical approaches have been attempted in recent years to evaluate antioxidant capacity (AOC) and the total polyphenols content (TP) in food samples (Della Pelle & Compagnone, 2018; Carocho & Ferreira, 2013). Particular emphasis has been given to assays having the advantage of sensitivity, rapidity, simplicity, cost effectiveness and reduced sample volume; this latter characteristic is related in particular to waste disposal. A key role for the development of novel AOC assays has been recently played by nanomaterials. Nanomaterial based AOC assays are valid alternatives to classical methods for polyphenols analysis, allowing rapid and smart assessment of food antioxidants. Recently, optical, electrochemical and bioelectrochemical nanomaterials-based approaches for AOC assay of food polyphenols have been reviewed by Della Pelle et al. (Della Pelle & Compagnone, 2018). Optical methods for AOC assays in food based on metal nanoparticles (MNPs) formation/aggregation mediated by reducing agents (Della Pelle & Compagnone, 2018; Vasilescu, Sharpe, & Andreescu, 2012; TułodzieckaSzyd & Szydłowska-Czerniak, 2016;

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Vilela, González, & Escarpa, 2012) as well as on quantum dots (Della Pelle & Compagnone, 2018; Vilela, González, & Escarpa, 2015) have emerged and successfully proposed.

The use of gold nanoparticles (AuNPs) was introduced as novel tool for reliable assessment of AOC (and for the evaluation of total polyphenolic content) in food and biological samples (Della Pelle et al., 2015; Della Pelle, Vilela, González, & Escarpa, 2015; Tułodziecka & Szydłowska-Czerniak, 2016; Vilela, González, & Escarpa, 2015). These methods are based on the reduction of metal ions (generally inorganic salts or metal complexes) to MNPs in colloidal dispersions form. Optical detection is achieved exploiting the MNPs localized surface plasmon resonance (LSPR), which refers to the collective oscillation of the conduction electrons of the metal (Della Pelle & Compagnone, 2018; Lopatynskyi, Lopatynska, Guo, & Chegel, 2011). The latter is one of the most remarkable features of MNPs; in fact, strong absorption band(s) or increased scattering intensity of the radiation occurs at certain wavelengths for the MNPs as a result of this phenomenon. LSPR of the MNPs is mainly related to the MNPs size, shape, composition, inter-particle distance, and dielectric constant (refractive index) of the surrounding medium (Della Pelle & Compagnone, 2018; Vilela et al., 2015; Lopatynskyi et al., 2011).

The synthesis of silver nanoparticles (AgNPs) using different reducing compounds and natural extracts has been also reported because of the great interest into the “green” synthesis of AgNPs used in nanomedicine and microbiological applications (Ahmad et al., 2010; Marambio-Jones & Hoek, 2010; Moulton et al., 2010; Sharma, Yngard, & Lin, 2009). However, the formation of AgNPs by natural antioxidants as index of the AOC of the sample been proposed only in few papers (Chen, Zhang, Cao, & Huang, 2013; Özyürek, Güngör, Baki, Güçlü, & Apak, 2012; Teerasong, Jinnarak, Chaneam, Wilairat, & Nacapricha, 2017). In all these works the assay is not so straightforward; in fact, the antioxidant capacity is assayed as the ability to reduce Ag^+ ion in order to grow AgNPs already generated by another reducing agent. Thus, the approach requires multiple incubation steps. A AgNPs method for the evaluation of AOC of rapeseed and its products was also proposed by Szydłowska-Czerniak et al. (Szydlowska-Czerniak, Tulodziecka, & Szlyk, 2012), AgNPs formation using sinapic acid (as reference compound) and samples extraction influence on the method were investigated carrying out the measurement at pH 8.4 after 60 min of incubation.

In this work a simple and rapid colorimetric method for the detection of polyphenols based on the direct reduction of Ag^+ ions to produce AgNPs is proposed. The single step assay realised is simple, rapid and highly sensitive. The ability of different classes of polyphenols to form AuNPs and AgNPs in different conditions was studied using 15 polyphenols and 9 different samples. Data were compared with classical methods for AOC assay demonstrating the feasibility of the proposed approach. In order to understand the influence of the samples, endogenous polyphenols composition onto the MNPs formation UHPLC–MS/MS analysis of the phenolic content of the samples has been also carried out.

2. Materials and methods

2.1. Reagents, stock solutions and samples

All the chemicals were of analytical reagent grade. Epicatechin, catechin, epigallocatechin, catechol and phlorizin were purchased from Extrasynthese (Genay, France). Quercetin, gallic acid, kaempferol, rutin, p-cumaric acid, ferulic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), caffeic acid, myricetin, clorogenic acid, were purchased from Sigma-Aldrich (St Louis, MO, USA). Cetyltrimethylammonium chloride (CTAC; 25% in water), Cetyltrimethylammonium bromide (CTAB), polyethylene glicol (PEG), ethylenediaminetetraacetic acid (EDTA), hydrogen tetracholoroaurate ($HAuCl_4{\cdot}3H_2O$, 99.9%), silver nitrate ($AgNO_3$, > 99%), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), sodium hydroxide (NaOH) and sodium carbonate (Na_2CO_3) were purchased from Sigma-Aldrich (St Louis, MO, USA). Folin–Ciocalteu reagent was obtained from Merck Schuchardt (Hohenbrun, Germany). Sodium phosphate monobasic monohydrate ACS reagent ($NaH_2PO_4{\cdot}H_2O$) sodium phosphate dibasic anhydrous (Na_2HPO_4) for buffer solution preparation, methanol, acetonitrile and formic acid were bought from Sigma-Aldrich (St Louis, MO, USA). Stock solutions of polyphenol standards (in methanol) were prepared at a concentration of 1.0×10^{-2} mol L^{-1}, and stored at −20 °C in the dark. Different kinds of samples were purchased in local markets: digestive infusion (DIG), fennel infusion (IN), lemon tea (LT), pink forest infusion (RB), relax infused (RE), sogni d’oro camomile (SD), classic tea (TC), green tea (TG) and vanilla tea (VT). Samples were treated as follows: 500 mg were extracted using 10 mL of methanol under stirring for one hour in the dark at room temperature, samples were then centrifuged for 10 min (5000 rpm) and surnatants were collected, filtered (0.45 μm, PTFE syringe filter) and stored in the dark at −20 °C.

2.2. Apparatus

The centrifugation and stirring steps have been performed with a 4218 centrifuge from ALC International (Milano, Italy) and a sample orbital shaker SSL1 from Stuart equipment (Belfast, UK) respectively. For the assays the samples were heated in a water bath using a thermostat digital group 720 D (Asal, Italy). Absorbance measurements were carried out using a JENWAY 6400 Spectrophotometer from Barlworld Scientific (Staffordshire, UK,). The nanoparticles were characterized by transmission electron microscopy (TEM) using (TEM, S-2400 N, HITACHI, Japan). The samples for TEM characterization were prepared by placing a drop of the dilute sample solution on a carbon-coated copper grid and dried at room temperature before measurements. The samples were analyzed using an UHPLC Nexera LC20AD XR from Shimadzu (Kyoto, Japan) equipped with autosampler, vacuum degasser, and column oven. The UHPLC detection has been performed with a triple quadrupole mass spectrometer 4500 Qtrap from Sciex (Toronto, ON, Canada).

2.3. Silver nanoparticles antioxidant capacity based assay

Two different kind of assay were used for AgNPs: at room temperature (AgNPs-*RT*) and at 45 °C (AgNPs-*HT*).

2.3.1. AgNPs-RT

AgNPs were produced in 500 μL volume (reaction in eppendorf) with final concentrations of 8.0×10^{-6} mol L^{-1} of CTAC, 2.5×10^{-4} mol L^{-1} of $AgNO_3$ and 1.0×10^{-1} mol L^{-1} of NaOH (final pH = 13.0). 5 μL of CTAC solution (8.0×10^{-4} mol L^{-1}) was initially added to NaOH diluted in deionizer water, then 25 μL of $AgNO_3$ solution (5.0×10^{-3} mol L^{-1}) and appropriate dilutions of polyphenols standard or sample (reducing agent). All reagents need to be at room temperature. The reaction mix was stirred for 10 min in an orbital shaker and the reaction was blocked in ice for 10 min (this allows measurements in series, absorbance can be also taken immediately). Absorbance spectra were recorded in the 350–800 nm range against blank (all the reaction mix without polyphenols).

2.3.2. AgNPs-HT

AgNPs were obtained in phosphate buffer (pH 8.4; 1.0×10^{-2} mol L^{-1}) added with 8.0×10^{-6} mol L^{-1} of CTAC and 1.0×10^{-3} mo L^{-1} of $AgNO_3$. 5 μL of CTAC (8.0×10^{-4} mol L^{-1}), 25 μL of $AgNO_3$ solution (2.0×10^{-2} mol L^{-1}) and appropriate dilutions of polyphenols standard or sample reducing agent (final volume was 500 μL also in this case) were added to phosphate buffer (final pH = 8.4). The solution was stirred for 2 min and heated for 10 min at 45 °C in a water bath. The reaction was blocked in ice for 10 min (in this