JOURNAL OF FOOD AND DRUG ANALYSIS XXX (2017) 1–8

Available online at www.sciencedirect.com

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journal homepage: www.jfda-online.com

Original Article

Silver nanoparticle biosynthesis by using phenolic acids in rice husk extract as reducing agents and dispersants

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ARTICLE INFO

Article history:
Received 29 April 2017
Received in revised form
19 July 2017
Accepted 21 July 2017
Available online xxx

Keywords:
Rice husk
Silver nanoparticles
Biosynthesis
Phenolic acid
Caffeic acid

ABSTRACT

Rice husk extract, obtained using acid and alkali pretreatment extraction (AAPE), contains bioactive compounds and exhibits reducing abilities. Phenolic composition in rice husk extract was analyzed and the mechanism of silver nanoparticle (AgNP) biosynthesis by using AAPE rice husk extract was investigated in this study. Stable and spherically shaped AgNPs with a size of <15 nm were prepared under the following conditions: 0.001 M $AgNO_3$, AAPE rice husk extract diluted 10 times, pH 10, and reacted at 25 °C for 60 min. Synergistic effects among phenolic acids contributed to the formation of AgNPs, with the acids acting as excellent reducing agents (owing to their abundant hydroxyl groups) and excellent dispersants (owing to their derived C=O groups), which enhanced the NPs' stability. Caffeic acid (CA) was demonstrated to synthesize AgNPs independently and is suggested to be the most crucial compound for reducing Ag^+ during the biosynthesis with rice husk extract. A possible mechanism and reaction process for the formation of AgNPs synthesized using CA in rice husk extracts is proposed.

1. Introduction

Silver nanoparticles (AgNPs), between 1 and 100 nm in size, have gained interest because of their distinctive physicochemical and optical properties and are employed in fields such as biological detection [1], conduction [2], catalysis [3], antimicrobials [4,5], and wound healing [6]. AgNPs can be prepared through various chemical and physical methods, such as through chemical reduction with sodium borohydrate as the reducing agent and by using capping agents to stabilize the solution and prevent flocculation. The disadvantages of these methods are typically that they require highly toxic chemicals or complex steps to achieve purification [7,8]. Biosynthesis of nanostructure materials is not only a favorable method of fabricating AgNPs but also an ecofriendly approach because it minimizes the use of substances that are hazardous to human health and the environment [9,10].

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http://dx.doi.org/10.1016/j.jfda.2017.07.005

In the search for a clean and nontoxic AgNP synthesis method, biosynthesis is a desirable candidate because it is analogous to the preparation of nanocrystals through chemical reduction. The raw materials used are biocompatible and reducible bioresources. Several studies have reported using natural material sources such as phenolics in plants, enzymes in microorganisms, and honey for synthesizing AgNPs [11–13]. Bioreduction of Ag^+ to yield metal NPs has been performed using various cereals, such as the bran of husk extract from *Oryza sativa* [13]; *Sorghum bicolor* [14]; and the seeds of *Macrotyloma uniflorum* [15].

The reduction reaction in AgNP biosynthesis is performed under mild temperature and pressure conditions, the metal precursor is used at a low concentration, and the nanometal product is stable [16]. Rice husk contains bioactive compounds, such as phenolic acids, which promote the antioxidant activity of rice in an antioxidant defense system [17,18]. Approximately 20% of paddy weight is the husk, which is discarded during the rice husking process and forms a major proportion of agricultural waste in Taiwan. Accordingly, the present study investigated the biosynthesis of AgNPs using rice husk extract as a reduction agent and stabilizer.

Phytochemicals are regarded as the major ingredients involved in the biosynthesis of AgNPs [7,15], and phenolic acids are the major phytochemicals in rice husk [19]. Phenolic acids have been reported to possess hydroxyl and carbonyl groups, which can bind to metals. Phenolic compounds may inactivate ions through chelation, and their chelating ability is likely related to the high nucleophilic characteristic of their aromatic rings [15]. Therefore, phenolic acids should have high antioxidant activity.

For most applications, the properties of metal NPs are determined by their size, shape, composition, and structure [20]. The preparation of high-quality AgNPs with controllable physicochemical properties is of great importance. Therefore, the effects of experimental parameters such as pH, incubation temperature, extract concentration, and $AgNO_3$ concentration on the formation of AgNPs were studied to identify desirable biosynthesis conditions when acid and alkali pretreatment extraction (AAPE) rice husk extract is used to obtain well-dispersed and stable AgNPs. The Ag^+ reduction process corresponded to a change in the structure of the phenolic acids, and the stabilization mechanism of AgNPs by phenolic acids was deduced in this study. The phenolic acids that contributed primarily to the reduction reaction during the biosynthesis were also analyzed.

2. Materials and methods

2.1. Materials and chemicals

Fresh rice (*O. sativa* japonica, Kaohsiung 145) husk was collected from the area surrounding Yilan County, Taiwan. The rice husk was hot-air-dried until its water content was less than 5% at 60 °C. The dried rice husk was dry-milled, and powder smaller than 60 mesh was collected as rice husk powder (RHP).

Sodium hydroxide, hydrochloric acid, methanol, and ether were purchased from Merck (Darmstadt, Germany). Ethyl acetate was purchased from Macron Chemicals (Center Valley, PA, USA). Folin–Ciocalteu reagent, Na_2CO_3, 4-hydroxybenzoic acid (4-hyd BA), 3-hydroxybenzoic acid (3-hyd BA), caffeic acid (CA), syringic acid (SA), gallic acid (GA), vanillic acid (VA), ferulic acid (FA), *p*-coumaric acid (*p*-Cou), $AgNO_3$, and reagents for preparation of buffer solution were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MS, USA). Protocatechuic acid (proto CA) and Ag powder (0.6–2 μm, 99.9%) were purchased from Alfa Aesar Co. (Ward Hill, MA, USA).

2.2. Extraction of phenolics from rice husk

For AAPE, phenolic acids were extracted using the method reported by Tang [21] with minor modifications. RHP was hydrolyzed with concentrated sulfuric acid under a 1:8 (w/v) ratio, and this mixture was heated for 40 min at 115 °C in a high-pressure reactor (Model 4522M, Parr Instrument Company, Moline, IL, USA). The cooled reaction product was filtered and the retentate was washed with deionized water to neutralize its pH, after which it was dried for 6 h at 50 °C to become acid-hydrolyzed rice RHP (AHRHP). The AHRHP was reacted with 8.7% sodium hydroxide under a 1:30 (w/v) ratio, and the mixture was heated for 118 min at 132 °C in a high-pressure reactor. The alkali-treated AHRHP solution was adjusted with concentrated hydrochloric acid to a pH of approximately 2.0–3.0 to avoid the pK_{a1} of the phenolic acids (approximately 4–5). The solution was extracted with 4 °C ether:ethyl acetate (1:1, v/v) and was shocked ultrasonically (LEO-150, Leo Ultrasonic Co., Ltd, New Taipei City, Taiwan) for 30 min. The mixture was then centrifuged (Model 7780, Kubota Corporation, Japan) at 2500 ×*g* for 15 min at 4 °C, after which the supernatant was collected. Sediment was extracted three times following the outlined procedure. These extracts were concentrated and blow-dried with nitrogen. The dried extracts were dissolved in methanol and used as AAPE solution.

For hot water extraction (HWE), RHP and deionized water (1:1, w/v) were heated for 30 min at 90 °C. The cooled crude extract was centrifuged at 8000 ×*g* for 30 min. The filtered supernatant was used as the HWE solution.

2.3. Determination of phenolics in rice husk

The total phenolic content was determined using the Folin–Ciocalteu method [22]. A total of 0.1 mL of the extract was placed in a test tube and then 0.50 mL of Folin–Ciocalteu reagent and 6.00 mL of deionized water were added. After incubation for 2 min, 2 mL of 15% Na_2CO_3 was added, after which the solution was left for 0.5 min; water was then added to bring the volume to 10.0 mL. Absorbance was measured through UV–vis spectrophotometry (U2001 UV–vis Spectrophotometer, Hitachi, Japan) at 755 nm after incubation for 2 h. The content of the phenolic compounds was analyzed according to the method of Tang [21] by using high-performance liquid chromatography.

2.4. AgNP biosynthesis conditions

The AgNPs were prepared with a 1:3 volume ratio of diluted AAPE rice husk extract to $AgNO_3$, subjected to 5 min of ultrasonic vibration, and then reacted in a water bath. Biosynthesis

Please cite this article in press as: Liu Y-S, et al., Silver nanoparticle biosynthesis by using phenolic acids in rice husk extract as reducing agents and dispersants, Journal of Food and Drug Analysis (2017), http://dx.doi.org/10.1016/j.jfda.2017.07.005