



Harnessing the wine dregs: An approach towards a more sustainable synthesis of gold and silver nanoparticles

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ABSTRACT

In recent years, the management of food waste processing has emerged as a major concern. One such type of food waste, grape pomace, has been shown to be a great source of bioactive compounds which might be used for more environmentally - friendly processes for the synthesis of nanomaterials. In this study, grape pomace of *Vitis vinifera* has been used for the obtainment of an aqueous extract. Firstly, the reducing activity, total phenolic content and DPPH scavenging activity of the aqueous extract were determined. Then, the aqueous extract was used for the synthesis of gold and silver nanoparticles. The formation of spherical and stable nanoparticles with mean diameters of 35.3 ± 5.2 nm for Au@GP and 42.9 ± 6.4 nm for Ag@GP was confirmed by UV–vis spectroscopy and transmission electron microscopy. Furthermore, the functional group of biomolecules present in grape pomace extract, Au@GP and Ag@GP, were characterized by Fourier transform infrared spectroscopy prior to and after the synthesis, in order to obtain information about the biomolecules involved in the reducing and stabilization process. This study is the first to deal with the use of *Vitis vinifera* grape pomace in obtaining gold and silver nanoparticles through an eco-friendly, quick, one-pot synthetic route.

1. Introduction

The huge imbalance between food production and consumption is well illustrated by the fact that, on average, 30–40% of food is wasted in many parts of the world [1]. Consequently, public attention has been increasingly focused on the limited capacity of local eco-environments to absorb the impact of food wastage [2]. In particular, fruit waste has been identified as a major environmental concern.

The global wine industry is responsible for a large proportion of worldwide grape production (*Vitis vinifera* L. subsp. *Vinifera*). During the winemaking process, grape pomace is the main waste material produced. Grape pomace (GP) consists mainly of peels (skins), seeds and stems, and it is estimated that this corresponds to 20–25% of the weight of the grape crushed for wine production.

Several studies have demonstrated that GPs are characterized by high contents of phenolics due to an incomplete extraction during the winemaking process. This means that the pomace extract may represent an eco-sustainable and low-cost source of natural antioxidants [3]. These phenolics are secondary plant metabolites with potential beneficial effects on humans. Therefore, GP constitutes an inexpensive source for the extraction of phytochemicals that can be used to benefit human health [4], cosmetic, and food industries [5]. Moreover, due to

their high antioxidant capability, GPs can also be used in the nanotechnology field.

Within the nanotechnology field there is a growing interest in the synthesis of novel nanomaterials based on the concept of green nanotechnology [6,7]. This integrates the principles of green chemistry and green engineering to produce eco-friendly, safe nanoparticles that do not use toxic chemicals in their synthesis protocol. In addition, the synthesis of metallic nanoparticles such as gold and silver are of great interest due to their unique physico-chemical properties and their applications in different fields, such as the food industry and in medicine [8,9]. Green nanotechnology offers an integrated approach to recycling, reuse or recovery of waste from the agri-food based industries.

Even though many researchers have produced metal nanoparticles from juice, seed, peel, raisins and leaf extracts of different varieties of *Vitis vinifera* [3,10–16], to the best of our knowledge, this is the first study of the synthesis of gold and silver nanoparticles led by grape pomaces. The pomace used was from the grape *Vitis vinifera* and the variety was Airén, a white grape commonly used in winemaking that is grown in Spain. In this study, the optimization of an eco-friendly and cost effective method for the synthesis of gold and silver nanoparticles using an aqueous extract grape pomace is described. This represents an important, sustainable use for grape wastage.

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2. Materials and Methods

All reagents used were of analytical grade and utilized without further purification in the assays. Milli-Q water was used where necessary throughout the experiments. Piranha solution (4:1 H₂SO₄/H₂O₂) and aqua regia (3:1 HCl/HNO₃) were used to clean all glassware and then rinsed thoroughly with water for complete removal of organic and metallic residues.

2.1. Preparation of Grape Pomace Extract

Grape pomace was kindly provided by a wine producer from Ourense, Spain (42°7'60" N 7°10'0" W). Grape pomace was stored in plastic bags at 4 °C until treatment. Once in the laboratory, the grape pomace was dried at 100 °C for 48 h, and ground to obtain a fine powder using a grinder. 10 g of the fine powder was then placed in a double neck balloon connected to a refrigerant. 100 mL of Milli-Q water was added and the mixture was then boiled at reflux for 1 h. Once the color had changed to brown, the flask was moved to an ice bath to complete the extraction. The extract was then centrifuged in a Beckman coulter Microfuge 16 at 4500 rpm for 10 min and the supernatant was filtered. Part of the extract obtained was stored at 4 °C and the remnant was frozen at -4 °C, until further treatment.

2.2. Characterization of Grape Pomace Extract

The reducing power and the content of phenols of grape pomace extract were measured following our modified version of the Oyaizu and Folin and Ciocalteu methods [17]. All trials were performed in triplicate and the results were expressed as mean ± standard deviation.

The free radical scavenging activity of the extracts on 1,1-diphenyl-2-picryl-hydrazyl (DPPH) was evaluated according to our previously reported method [18]. In summary, a 0.1 mM solution of DPPH in methanol was freshly prepared and 1 mL of this solution was added to 3 mL of the aqueous extracts diluted at a ratio of 1:12. The mixtures were shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm using a UV-vis spectrophotometer. Ascorbic acid was used as the reference (at a concentration range of 2–0.1 mg/L). For blank, 3 mL distilled water was used instead of the sample and a sample control was also made for each fraction by mixing 3 mL of sample with 1 mL of methanol. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability of scavenging the DPPH radical was calculated by using the following formula.

$$\text{DPPH scavenging effect (\%inhibition)} = \left(1 - \frac{A_s - A_{s0}}{A_b}\right) \times 100$$

where A_b is the absorbance of the blank, and A_s is the absorbance in the presence of the extract samples and reference, and A_{s0} is the sample control. All the tests were performed in triplicates and the results were averaged. The effective concentration, which required the decrease of the initial DPPH concentration by 50% (EC50), was also calculated.

To obtain information about the main components in the grape pomace aqueous extract, Gas Chromatography-Mass Spectroscopy (GC-MS) was performed. A Hewlett-Packard 5890IiplusGC system with a split/splitless injector and interfaced with a Hewlett-Packard 5989^a mass spectrometer analyzed the sample. The injector was set at 280 °C. For separation, an Agilent 19091J-436 HP-5 5% Phenyl Methyl Siloxane column (60 m × 0.25 mm × 0.25 μm) was used. Helium was the carrier gas (1.1 mL/min). Three microliters of the sample were injected. The GC conditions were listed as follows: initial temperature was 35 °C for 10 min, increasing at 5 °C/0.5 min up to 325 °C and held for 15 min to give a total run time of 83 min. The acquisitions were undertaken in scan mode (from 30 to 600 amu). Peak identification was

Table 1
Different reaction conditions tested for the synthesis of Au@GP.

mL GPE/mL H ₂ O	[Au] mM
1:0	0.35
1:1	
1:3	
1:4	
1:3	0.125
	0.188
	0.25
	0.31
	0.35
1:4	0.20
	0.25
	0.30
	0.35
	0.40

carried out by analogy of mass spectra with those of the mass library (WILEY 6.0 and NIST 2.0).

2.3. Preparation of Gold Nanoparticles Au@GP

For the synthesis of gold nanoparticles, different concentrations of GP extract and HAuCl₄ were tested. In summary, different volumes of HAuCl₄ 0.01 M were slowly added to the GP extracts; the solution was kept at room temperature while stirring until color change. The evolution of the reaction was monitored by UV-vis spectroscopy. The different conditions tested are shown in Table 1.

2.4. Preparation of Silver Nanoparticles Ag@GP

For the synthesis of silver nanoparticles, different concentrations of GP extract and AgNO₃ were tested. The extract was heated until ebullition. Then different volumes of AgNO₃ 0.005 M were added. The evolution of the reaction was monitored by UV-vis spectroscopy. The different conditions tested are shown in table 2.

2.5. Characterization of Au@GP and Ag@GP

UV-vis spectra were recorded at room temperature on a Jasco Spectrometer V-670. A Jasco FT/IR-6100 spectrophotometer was used at a resolution of 4 cm⁻¹ in the range of 4000–400 cm⁻¹. For the preparation of the samples for FTIR measurements, GP extract, Au@GP and Ag@GP were freeze dried. The dried materials were ground to fine powder and used to record the spectra employing the KBr pellet technique. Transmission electron microscopy (TEM) samples were prepared by dropping the nanoparticles directly onto a Formvar-carbon coated copper grid. TEM images were acquired with a JEOL JEM1010 TEM, operated at 100 kV. All acquisition and data analysis were carried out using Digital Micrograph software by Gatan.

Table 2
Different reaction conditions tested for the synthesis of Ag@GP.

mL GPE/mL H ₂ O	[Ag] mM
1:4	0.250
1:9	
1:9	0.125
	0.250
	0.375

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