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Enzymatic browning reduction in white cabbage, potent antibacterial and antioxidant activities of biogenic silver nanoparticles

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ABSTRACT

To produce economically benign and nontoxic enzymatic browning reducing and antimicrobial agents is the goal of nanotechnology. In this study we synthesized environmentally friendly silver nanoparticles (AgNPs) using Longan fruit juice as reducing and stabilizing agent. Silver nanoparticles synthesis was monitored by UV–vis spectroscopy showing localized surface plasmon resonance at 443 nm. XRD (X-ray diffraction analysis), HRTEM (high resolution transmission electron microscopy) and EDX (energy dispersive X-ray analysis) were used to characterize crystalline structure, size (4–10 nm), shape and elemental composition of silver nanoparticles. Surface capped phytochemicals were characterized by FTIR. Silver nanoparticles have significant enzymatic browning reduction ($p < 0.001$) using white cabbage as a model system. No research has been reported on the Enzymatic browning reduction of biosynthesized silver nanoparticles. The silver nanoparticles showed prominent antibacterial activity with MIC values of 31.25 μg/ml against *Staphylococcus aureus* and *Basillus subtilus* while 62.5 μg/ml against *Escherichia coli*. High cells constituents' release was exhibited by *B. subtilus* with 2× MIC value of silver nanoparticles. Silver nanoparticles exhibited no hemolytic activity against Red blood cells of male Wistar albino rat. Silver nanoparticles also showed significant DPPH free radical scavenging activity. This research would have an important implication for the synthesis of more efficient antimicrobial, antioxidant and anti-enzymatic browning agents for food preservation, processing and other biomedical applications.

1. Introduction

To preserve the color of fruit juice during processing and storage is one of the key objectives of fruit processors because changes in the structure of fruit products change the color and final appearance of the product. Browning is one of the main factors that can change the color of fruit juice and limits its' commercial shelf life [1,47]. Therefore browning needs to be controlled during the processing stages of the food to preserve their quality, beyond this the organoleptic and nutritional properties will be strongly changed. For these reasons and due to the importance of appearance as a quality parameter, the prevention of these undesirable reactions has always been a challenge for food scientists [47,35].

Cabbage (*Brassica oleracea* L. capitata) is a versatile food and is increasingly becoming an important vegetable in restaurants, dinning commons and fast food outlets because of its ease and less requirement for washing, cutting or shredding [21]. However, cabbage and a variety of fruits and vegetables, such as lettuce, potato, apple and banana are susceptible to enzymatic browning during processing and storage. Enzymatic browning in vegetables is often associated with undesirable brown colors, off-flavors and lower nutritional value [42]. The browning reaction needs the presence of oxygen, polyphenol oxidase (PPO) and phenolic compounds and is generally triggered by the enzymatic oxidation of monophenols into o-diphenols and quinones, which further undergo non-enzymatic polymerization leading to the formation of pigments [36]. Although enzymatic browning is beneficial to the color and flavor development of certain food items such as tea and coffee but it impairs the quality of fresh-cut produce [27]. About 30% loss of quality in post-harvest food supplies including vegetables and fruits in developing nations is due to enzymatic browning [20,34]. In recent decades, several methods have been used to prevent PPO activity in foods [26,44,47,45,53,55]. However, U.S. Food and Drug Administration (FDA) has restricted the use of sulfites to inhibit the browning of foods, because they have been associated with severe allergy-like reactions in certain populations [11,44]. Moreover, heat treatments are not suitable for inhibiting this sort of reaction, and so several other methods

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have been tried to reduce PPO activity, including the addition of ascorbic acid or chemical agents, the exclusion of oxygen, refrigeration and various non-thermal treatments [29,51,52]. Similarly honeys can also be used for enzymatic browning reduction [33].

Additionally for many decades, food borne ailments have been also considered as serious threats to public health all over the world. In food borne pathogens studies, four major pathogens have emerged significantly important in terms of human health and diseases. These include: *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium* and *Vibrio parahaemolyticus*. These organisms have commonly been related with food products and associated to a number of human diseases [59]. *E. coli* O157: H7 is a major worldwide cause of diarrhea, hemorrhagic colitis and hemo-lytic-uremic syndrome. The disease is often associated to the use of infected and undercooked ground beef as well as unpasteurized fruit juices (A. K. [46,48]). *L. monocytogenes* has been implicated in food borne outbreaks and subsequently isolated from various products. Such as meat, milk, milk products, vegetables, poultry and fish [12].

In this respect, there has been a renewal of interest in anti-browning agents and inhibitors of microorganisms causing food spoilage, which are cheap, economically benign, nontoxic and eco-friendly. Among such reagents are the phytochemicals based silver nanoparticles [2,4,19]. Plants have been known to internally bio-mineralize calcium carbonate, silica and even magnetite. Certain plants are known to hyper-accumulate these heavy metals within different parts of plants. The internal accumulation of metals in plants can occur both *via* complexation of the metal ion with a suitable bio-ligand in its native oxidation state or after it's reduction to a lower oxidation state. The possibility of reduction of metal ions by plants and the presence of metal complexing agents in them entices a materials scientist to use plants for the purpose of synthesizing nanoparticles and controlling their size and shape and to test the possibility of synthesizing nanoparticles of low reduction potential metals [3]. Due to the improvement of antibiotic resistance in pathogenic bacteria, the pharmaceutical companies and researchers are searching for novel antimicrobials and the AgNPs are capable candidate for the same. The reduction of Ag^{+} ions leads to the formation of silver atoms (Ag) which is followed by agglomeration into oligomeric clusters. These clusters eventually lead to the formation of colloidal AgNPs. When the colloidal particles are much smaller than the wavelength of visible light, the solution possess characteristic yellow color with an intense band in the 380–430 nm range and other less intense bands at longer wavelength in the UV–visible absorption spectrum.

Silver nanoparticles can act as anti-browning agent, antioxidant, reducing agent and inhibitors of food borne pathogens. Here in we report the phytochemicals inspired Ag nanoparticles using Longan fruit juice as reducing, capping and stabilizing agent. These nanoparticles are studied for their Enzymatic browning reduction in white cabbage, antioxidant (DPPH free radical scavenging), antibacterial activities against food borne pathogens and Hemolytic activity against healthy RBCs of Wistar albino male rat.

2. Materials and methods

2.1. Materials

Longan fruit and white cabbage were purchased from the local market. $AgNO_3$, Vit. C, DPPH, Nutrient agar, nutrient broth and methanol were purchased from Beijing chemical works, China. All solutions were made in sterile double distilled (DD) water and methanol. All apparatus were washed with aqua regia and rewashed with DD water.

2.1.1. Juice extraction

Longan flesh juice was quenched using juice extractor. The juice was centrifuged at 10,000 rpm for 10 min at 4 °C to remove the solid fruit material. The supernatant was used as a reducing, capping and stabilizing agent of Ag nanoparticles.

2.1.2. Syntheses

25 ml Longan fruit juice was mixed with 75 ml of 6 mM aqueous solution of $AgNO_3$ in 250 ml flask to synthesize Ag nanoparticles. The mixture was magnetically stirred at room temperature. Ag nanoparticles were separated from the colloidal solution by repeated centrifugation at 12,000 rpm for 10 min and 4 °C. Then the Ag nanoparticles were freeze dried using VirTis freeze mobile 6ES freeze drier.

2.1.3. Characterization

The biosyntheses of Ag nanoparticles was monitored frequently by scanning the aliquot sample in the wavelength range of 350–800 nm in Shimadzu UV-2450 spectrophotometer. The XRD measurements were examined by Rigaku Miniflex X-ray diffracto meter. A Hitachi EDX elemental microanalysis system and JEOL3010 high resolution transmission electron microscope were used to determine the crystalline nature, morphology, size and elemental analysis of the Ag nanoparticles. Infra red (IR) spectrum was obtained using the KBr pellet technique on an ABB MB3000 spectrophotometer.

2.2. Ability of Ag nanoparticles to reduce enzymatic browning

The ability of Ag nanoparticles to reduce enzymatic browning was determined with a spectrophotometric assay [8] with a little modifications. 2 g of fresh homogenates of white cabbage were taken in separate test tubes and added 5 ml of double distilled (DD) water. Test samples were incubated for 1 h with and without Ag nanoparticles. Ag nanoparticles concentration varied from 1 mg to 0.125 mg/g (1, 0.5, 0.25 and 0.125 mg) of total homogenate. After incubation, an aliquot of each homogenate was extracted with aqueous methanol. The extract was centrifuged at 11,200 rpm for 5 min and 4 °C then the supernatant was passed through a 0.22 μm Teflon filter before conducting the spectroscopy. The absorbance was monitored at 420 nm against an aqueous methanol blank. Enzymatic browning reduction was presented in browning units, where a difference of 0.01 absorbance unit from control per gram homogenate was considered to be equivalent to 1 browning unit. Therefore, samples with the highest numbers indicate the greatest enzymatic browning reduction.

2.2.1. Micro organisms

Three food-borne pathogens including *E. coli* ATCC 8739 (Gram negative), *Staphylococcus aureus* ATCC 6538 and *Basilus subtilus* ATCC 6633 (Gram positive) were used in antimicrobial assay. These strains were maintained on agar slants at 4 °C in the College of life science and technology Beijing University of chemical technology, Beijing for antimicrobial tests. Micro organisms were incubated overnight at 37 °C in Mueller-Hinton Broth (Oxoid) at pH 7.4. Cephalexin (50 μl of 4 mg/ml) in sterile DD water was used as reference drug.

2.2.2. Antimicrobial screening

2.2.2.1. Screening for antibacterial activity. The antibacterial activity was determined through agar well diffusion method [16]. All bacterial strains were grown in nutrient broth at 37 °C for 24 h incubation till turbidity became equivalent to McFarland 0.5 turbidity standard. The inocula of the *B. subtilus*, *S. auroeus* and *E. coli* were streaked on to the condensed Muller Hinton agar (Oxoid) in petri plates by a sterilized cotton swab in order to make sure an uniform thick lawn or layer of growth following incubation. Wells of 8 mm in diameter were formed with the help of sterilized cork borer on to nutrient agar plates. The silver nanoparticles (50 μl of 4 mg/ml) in sterile DD water were put into the wells and the plates were allowed to stay for 2 h at room temperature. Finally, the plates were incubated at 37 °C for 20–24 h and the resulting