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Development of silver/titanium dioxide/chitosan adipate nanocomposite as an antibacterial coating for fruit storage

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ABSTRACT

A novel nanocomposite of silver/titanium dioxide/chitosan adipate (Ag/TiO_2/CS) was developed through photochemical reduction using a chitosan adipate template. Chitosan served as a reducing agent anchoring the metal ions through Ag–N coordination bonds and electrostatic attractions, thus stabilizing the Ag/TiO_2/CS product observed by Fourier transform infrared spectroscopy and X-ray diffraction patterns. Scanning electron microscopy observations revealed that the nanocomposite particles (50–100 nm) were deposited onto the chitosan adipate layer. The product exhibited high ζ-potentials from 30.1 mV to 33.0 mV during 60 days of storage. In addition, the nanocomposite demonstrated higher antimicrobial activity than $AgNO_3$ or nano-Ag particles at similar concentrations, as evidenced by the inhibition zone, minimum inhibitory concentrations (MIC), and growth curve. The nanocomposite reduced the *Escherichia coli* population by 6 logs after 24 h of incubation, and had an MIC value of 0.38 μg Ag/mL. These results suggest that Ag/TiO_2/CS has the potential to be used as an antibacterial protective coating for fruit storage.

1. Introduction

Chitosan is a biodegradable polysaccharide composed of N-acetyl-D-glucosamine and D-glucosamine, generated by the alkaline deacetylation of chitin (Onishi & Machida, 1999). It is the second most abundant natural biopolymer and relatively inexpensive (Ma et al., 2008). In addition, it is recognized as a nontoxic, biodegradable, biocompatible and environmentally-friendly material. Many properties that are beneficial for a protective fruit coating, including film forming capability and antibacterial effects (Vásconez, Flores, Campos, Alvarado, & Gerschenson, 2009). Nevertheless, chitosan has a few drawbacks, such as pH-dependent solubility, which can be overcome by using chitosan salts (Belalia, Grelier, Benaissa, & Coma, 2008), and high viscosity, which makes the coating slow to dry and difficult to attach in some gaps on the surface of fruits (Lin et al., 2011) thus impeding its antibacterial ability. In addition, fruits produce moisture and gaseous metabolites continuously during storage due to respiration. Respiration rates depend on storage temperature, time since harvest, commodity and condition (damaged vs intact). Chitosan coatings on the surface of fruits may initially be wet and sticky, which could discourage sales of coated fruits. Furthermore, despite initial wetness, chitosan coatings become dry and develop cracks over time. Therefore, chitosan or chitosan-based coatings have only limited short-term antibacterial effect (Geisberger et al., 2013).

Silver nanoparticles are of particular interest due to their unique properties, like the broad-spectrum of bactericidal activities, which can be incorporated for long-term antibacterial performance as non-edible coatings or additives in food packaging (Reidy, Haase, Luch, Dawson, & Lynch, 2013). Although excessive oral consumption of silver may cause acute immunological response or chronic argyria, the amount of silver that could migrate from the composite to the fruit surface and thus be ingested should be well below the lowest reported effect level of 0.5 mg/kg of bw/day in animal studies (Park et al., 2014) and well below levels typically found to cause toxic effects in humans (Hadrup & Lam, 2014). Silver and silver colloids have numerous applications in various fields because of their broad spectrum antimicrobial activity. The toxicity of silver nanoparticles depends on particle size. Particles sizing 15 nm and 30 nm have higher toxicity than those sizing 55 nm particles, and they were shown to have a slight cytotoxic effect on eukaryotic cells at concentrations greater than or equal to 75 μg/mL (Carlson et al., 2008).

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In this study, the 50–100 nm nanoparticles were effective at inhibiting *Escherichia coli* cells at an MIC of 0.38 μg/mL, thus allowing a safe concentration despite potential particle migration. Many techniques have been investigated for the production of silver nanoparticles, including chemical reduction, electrochemical reduction, photochemical reduction, gamma-ray irradiation, UV irradiation, ultrasonic method, and microwave method (Byeon & Kim, 2012; Hettiarachchi & Wickramarachchi, 2011; Nadagouda, Speth, & Varma, 2011; Spadaro, Barletta, Barreca, Curro, & Neri, 2010). Various stabilizers have been used in these processes to achieve the desirable particle size, size distribution, shape, stability, and solubility of silver nanoparticles. The most commonly used stabilizers are synthetic polymers such as polyvinyl pyrrolidone (PVP), polyvinyl alcohol (PVA), polyaniline, and polyethylene glycol (PEG) (Bouazza, Alonzo, & Hauchard, 2009; Tan, Dai, Li, & Zhu, 2003). Additionally, natural polymers such as starch (Hu, Wang, Wang, Zhang, & Yu, 2008) and chitosan (Hettiarachchi & Wickramarachchi, 2011) have been studied as potential stabilizers because of their biocompatibility and biodegradability. Incorporation of metal ions into chitosan tripolyphosphate nanoparticles was found to enhance antimicrobial activity (Du, Niu, Xu, Xu, & Fan, 2009).

Titanium dioxide (TiO_2), a metal oxide semiconductor, is a promising photocatalyst due to its desirable catalytic efficiency, chemical stability, low toxicity, and acceptable cost (Egerton & Tooley, 2004; Tong, Binh, Kelly, Gaillard, & Gray, 2013). Nano-TiO_2 exhibits good adhesiveness and bactericidal activities and has been used as a generally recognized as safe (GRAS) additive in medical devices and biomaterials (Kochkodan, Tsarenko, Potapchenko, Kosinova, & Goncharuk, 2008). However, the commercial applications of nano-TiO_2 are limited due to its unstable thermodynamic properties and tendency to agglomerate. The photocatalytic activity of nano-TiO_2 is activated by UV light in the presence of oxygen and water molecules, and it results in the production of reactive oxygen species and hydroxyl radical that are responsible for the bactericidal function (Banerjee, Gopal, Muraleedharan, Tyagi, & Raj, 2006). Thus UV light exposure is necessary for nano-TiO_2 to exert strong antibacterial activity, beyond that achieved with either UV exposure alone or nano-TiO_2 alone (Banerjee et al., 2006). Titanium dioxide coating on substrates is considered to be a contact-active antibacterial agent because it kills bacteria in close proximity efficiently without releasing biocides, and its antibacterial performance is restricted to the coated surface (Yuan, Ji, Fu, & Shen, 2008).

The objective of this study is to construct an antibacterial coating of Ag/TiO_2/CS for the storage of fruits with rough skin, such as cantaloupes and lychees, to reduce bacterial proliferation in the cracks and pores. In order to enhance the water solubility of chitosan, we used adipic acid to produce chitosan salts. Such products are suitable for use in fruit coating because they do not cause tissue damage. The TiO_2 nanoparticles were incorporated into the chitosan adipate solution and silver ions supplied by $AgNO_3$ solution were then loaded onto the formed template and reduced in-situ, forming silver deposited TiO_2 nanocomposite particles. The nanocomposite Ag/TiO_2/CS product was characterized in terms of ζ-potential, particle size, surface charge, FTIR spectrophotometry, X-ray diffraction patterns, and scanning electron microscopy (SEM). The antimicrobial activity of Ag/TiO_2/CS was tested against non-toxigenic *E. coli* O157:H7.

2. Materials and methods

2.1. Materials and reagents

Chitosan with 77% deacetylation and medium molecular weight and TiO_2 nanoparticles with average diameter of 21 nm were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade.

2.2. Sample preparation

The first step in preparing the Ag/TiO_2/CS was the modification of chitosan to form its adipate salt (Lin, Wang, Li, Liang, & Du, 2012). A mixture of chitosan and adipic acid (-NH_2:-COOH = 1:1.2, molar ratio) was ground in a mortar using a pestle under semidry condition at room temperature until the mixture was water-soluble. The chitosan adipate product was washed using ethanol, dried at 50 °C for 2 h, and ground into powder.

A chitosan adipate aqueous solution (10 mg/mL, 2 mL) was prepared and mixed with $AgNO_3$ solution (10 mg/mL, 5 mL). The mixture was stirred magnetically for approximately 1 h in a water bath at 50 °C. Meanwhile, 0.10 g of TiO_2 particles were dispersed in 20 mL of distilled water in an ultrasound bath for approximately 10 min. Subsequently, a 200 μL aliquot of the TiO_2 dispersion was added into the Ag/chitosan adipate solution, which was then incubated at 50 °C for 0.5 h in the water bath. The solution was treated with UV radiation (15 W/m^2 = 365 nm) for 10 min to produce the Ag/TiO_2/CS nanocomposite. The Ag/CS and TiO_2/CS control samples were prepared under the same conditions as Ag/TiO_2/CS nanocomposite except that the Ag/CS and TiO_2/CS samples did not contain TiO_2 and $AgNO_3$, respectively.

Fig. 1 shows the schematic for the preparation of Ag/TiO_2/CS by UV irradiation (Lu et al., 2011). The conduction band of TiO_2 is represented by CB and VB. Under ultraviolet exposure, the silver ion (Ag^+) in $AgNO_3$ is reduced to elemental silver (Ag) in the presence of TiO_2 nanoparticles as a catalyst. Ultraviolet radiation facilitates electron transfer from TiO_2 to Ag^+ where it is stored temporarily in the conduction band of TiO_2 for Ag^+ reduction. The resulting Ag is deposited on the surface of the TiO_2 nanoparticles; meanwhile, it forms a complex with chitosan via Ag–NH_3 coordination bonds through the amino groups on chitosan.

2.3. Determination of ζ-potential and particle sizes of nanocomposite

The particle size was determined by dynamic laser scattering (DLS) using a BI-200 SM Goniometer Version 2 (Brookhaven Instrument Corp., Holtsville, NY). Particle dispersions in deionized water were measured without dilution. The refractive index and viscosity of water were applied to obtain accurate results. The laser power and aperture size were set consistently at 10 mW and

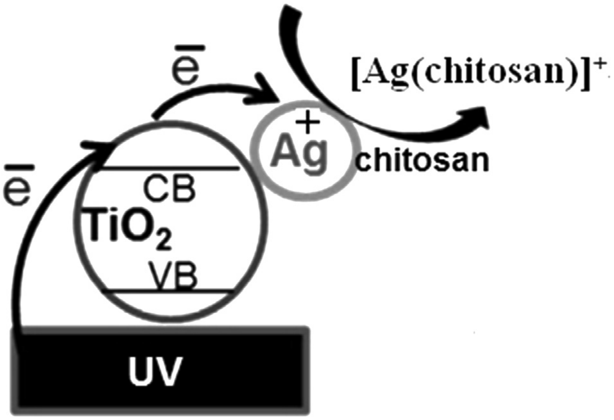

Fig. 1. Schematic view of the effects of UV irradiation on the formation of Ag/TiO_2/CS nanoparticles. CB and VB refer to conduction and valance bands respectively.