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Vitamin D-fortified chitosan films from mushroom waste

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ABSTRACT

Brown mushroom (*Agaricus bisporus*) stalk bases from mushroom waste were treated with UV-B light to rapidly increase vitamin D_2 content. Chitin was also recovered from this waste and converted into chitosan by *N*-deacetylation. FTIR spectra showed that the mushroom chitosan were similar to chitosan from animal sources. Chitosan films were prepared using high molecular weight (HW), low molecular weight (LW) and fungal chitosan. UV-B treated mushroom particles were also incorporated into fungal chitosan films. The fungal chitosan films showed similar density, porosity and water vapor barrier properties to the LW and HW chitosan films. However, fungal chitosan films were more hydrophobic and less flexible than the LW and HW chitosan films. Addition of mushroom particles did not significantly affect mechanical or water barrier properties of the fungal chitosan films.

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1. Introduction

The National Center for Health Statistics found that more than 70% of American adolescents and adults were deficient in Vitamin D in 2004 (Ginde, Liu, & Camargo, 2009). The major health problems resulting from vitamin D deficiency are rickets in children and osteomalacia in adults. Recently, there has been intense interest in the role of vitamin D in a variety of nonskeletal medical conditions. For instance, vitamin D has been linked to a significant reduced risk of breast cancer, colon cancer, prostate cancer, autoimmune disease and cardiovascular disease (Holick, 2004). Vitamin D intake comes naturally from exposure to sunlight since humans are capable of converting cholesterol present in the epidermis to vitamin D_3 (Holick et al., 1980). However, sunlight exposure might be inadequate to produce the amount of vitamin D required to maintain good health. Consequently, other sources of vitamin D, such as foods, are required to supplement those derived from sunlight.

The Food and Nutrition Board of the Institute of Medicine recommends a dietary allowance (DRA) for vitamin D of 600–800 IU (15–20 μg) per day (Institute of Medicine, 2010). Vitamin D exists in two distinct forms: vitamin D_3 (cholecalciferol), present mainly in oily fish and bovine liver, and vitamin D_2 (ergocalciferol), present in yeasts and mushrooms (Shrapnel & Truswell, 2006). Commercial mushroom growers have recently incorporated UV light into their production processes to enhance the vitamin D content of mushrooms from almost nondetectable levels up to 6000–8000 IU/g (150–200 μg/g). Following UV light exposure, ergosterol (the most abundant phytosterol in mushrooms) undergoes photolysis to produce previtamin D_2, which then slowly isomerizes to vitamin D_2 via a thermal reaction (Phillips et al., 2011; Villares et al., 2014). Vitamin D_2 produced from UV irradiated mushrooms has been shown to be bioavailable, with an increase in blood levels of 25-hydroxyvitamin D, which is a biomarker for a person's vitamin D status. This increase is similar to those from someone ingesting fortified food, vitamin D_2 supplement and pharmaceutical formulation (Keegan, Lu, Bogusz, Williams, & Holick, 2013; Koyyalamudi, Jeong, Song, Cho, & Pang, 2009; Urbain, Singler, Ihorst, Biesalski, & Bertz, 2011). Also, mushrooms represent a potential source of vitamin D that is not derived from animals and can be considered vegan.

Mushrooms can also be considered as an alternative non-animal source for chitosan production. Chitin and its derivative, chitosan, are abundant and renewable biopolymers found in nature. Chitosan is a natural, biocompatible and biodegradable polycationic polysaccharide that possesses antimicrobial activity and filmogenic properties. Chitosan-based films have shown antimicrobial

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activity, for instance, chitosan nanocomposite films loaded with silver and zinc oxide nanoparticles as well as bionanocomposites based on chitosan/poly(vinylalcohol)/titanium nanoparticles and chitosan/calcium silicate nanocomposites doped with $AgNO_3$ have shown good antimicrobial activity against gram positive, gram negative and fungi (El-Nahrawy, Ali, Abou Hammad, & Youssef, 2016; Youssef, Abou-Yousef, El-Sayed, & Kame, 2015; Youssef, El-Sayed, Salama, El-Sayed, & Dufresne, 2015). Currently, most of the commercial chitosan is derived from crustaceans, such as shrimps and crabs. However, mushroom cellular walls have high chitin content, which might be transformed into chitosan through a deacetylation reaction. Several mushrooms genera including *Agaricus, Pleurotus, Ganoderma* and *Lentinula* have been suggested as alternative chitosan sources (Kannan, Nesakumari, Rajarathinam, & Singh, 2010; Ospina et al., 2015; Yen & Mau, 2007)

Moreover, mushroom industry waste might be utilized to produce vitamin D and chitosan rather than using high-quality mushrooms (Wu, Zivanovic, Draughon, & Sams, 2004). During harvesting of mushrooms, their stalk bases are generated as a waste product. These bases comprise approximately 25% to 33% of the weight of fresh mushrooms and are normally used as low-economic value animal feed and compost (Chou, Sheih, & Fang, 2013).

Our hypothesis is that brown mushroom (*Agaricus bisporus*) waste can be used to obtain chitosan and develop nutritionally fortified films. In this study, we extracted chitosan from the stalk bases of brown mushrooms. We compared physicochemical properties of fungal chitosan with high molecular weight (HW) and low molecular weight (LW) chitosan from animal origin. We treated the mushroom stalk bases with UV-B light to increase vitamin D_2 content. We then cast and characterized HW and LW chitosan films as well as fungal and fungal chitosan films containing UV treated mushroom particles.

2. Experimental

2.1. UV treatment of mushrooms stalks

Brown mushroom (*Agaricus bisporus*) byproducts were obtained from Monterey Mushrooms, Inc. (Watsonville, CA) on the day of mushroom harvest. Mushrooms were medium sized, with cap diameters typically ranging from 3 to 4.5 cm as designated by the facility. Each batch of mushrooms was harvested in the morning by trained individuals. Mushroom stalks were collected by cutting off the mycelium from the stalk bases. Stalks were then stored at 5 °C overnight. One day after harvesting, the stalks were washed, cut into pieces and treated with UV-B radiation.A Uvitron UV Conveyor 40 dual-lamp curing system with two SunRay 400 SM UVB flood lamps (Uvitron International Inc., West Springfield, MA, USA) was used for the UV treatment. UV-B dose (energy) and peak intensity (power) were measured using an Uvicure Plus II radiometer (EIT Inc., Sterling, VA). The lamp height was set at 3.8 cm to achieve a peak intensity of 492 W/m^2. The conveyor speed was adjusted to achieve 1 J/cm^2 UV-B doses. During this experiment, the mushrooms were placed on the conveyor without regard to mushroom orientation and in a manner identical to that employed during a typical commercial production run.

After the UV-B treatments, mushroom stalks were placed flat in single layers on stainless steel trays and freeze dried in a Labconco Freezone 12 Freeze Dry System (Kansas city, MO, USA). After drying, the samples were ground to a fine powder with particle sizes smaller than 0.06 mm (Tyler screen size 28) and sealed under nitrogen in laminated PET bags (Impak corp., Los Angeles, CA, USA) for further analysis.

2.2. Vitamin D_2 analysis

The vitamin D_2 analysis was performed in agreement with the official AOAC 2002.05 method (AOAC, 2007) comprising alkaline hydrolysis (saponification) of the material with subsequent extraction of the hydrolysate with 40% ethanol and *n*-heptane and HPLC analysis using vitamin D_3 as internal standard. The HPLC analysis was performed using a Prominence HPLC system (Shimadzu, Kyoto, Japan) with an Inertsil ODS-P C-18 RP column (GL Sciences, Japan), 250 × 4.9 mm I.D., and particle size 5 μm, flow rate 1 ml/min, injection volume 50 μl, column temperature 40 °C, eluents: A (75% acetonitrile, 25% methanol) and B (100% ethanol). The gradient program was as follows: 0–11 min, linear gradient from 100% to 90% eluent A; 11–15 min, linear gradient from 90% to 20% eluent A; 15–19 min, linear gradient to 100% eluent B; 19–24 min, 100% eluent B; 24–32 min, linear gradient back to 100% eluent A. The detection was conducted using a SPD-M20A photodiode array detector at 264 nm. The vitamin D_2 peak was identified by comparison with authentic sample (Acros Organics, USA) and quantified using an internal standard of 10 μg vitamin D_3 (Acros Organics, USA).

2.3. Chitin extraction

Dried and ground mushroom stalk bases (100 g) were homogenized in 96% ethanol. The supernatant was separated by centrifugation at 10,000 rpm for 15 min (Dupont Sorvall centrifuge RC-5C, Thermo Fisher Scientific, Waltham MA, USA), and the extraction-centrifugation cycle was repeated two more times. Then sodium metabisulfite solution (1000 ml of 0.5% $Na_2S_2O_5$ dissolved in 5% HCl) was added to the mushroom residue and left for 1 h at room temperature (23 °C). Afterwards, the residue was washed with deionized water by centrifugation until it reached a pH of 7. The mushroom residue was mixed with 1000 ml of 2% NaOH and heated at 56 °C for 2 h. Then the mixture was centrifuged and the remains were washed 3 times with deionized water.

Sodium hydroxide solution (750 ml of 0.1 M NaOH) was added to the insoluble phase and then H_2O_2 was added gently to a final concentration of 3%. The mixture was continually stirred at 45 °C for 0.5 h. Crude chitin was obtained after centrifugation and washing until it reached a pH of 7

2.3.1. Chitin deacetylation

An aqueous solution of sodium hydroxide (400 ml of 50% NaOH) was added to the extracted chitin and the mixture was boiled for 2 h at 104 °C. Deionized water was continuously added to the original volume during the boiling process. Afterwards, the mixture was cooled to room temperature and the solid phase was separated by centrifugation at 10000 rpm for 15 min. Then HCl solution (1 M) was added until the solution reached a pH of 8.5. The precipitated chitosan was collected by centrifugation at 10,000 rpm for 15 min, washed with deionized water until it reached a pH of 5.7 and dried by lyophilization. As a comparison to mushroom chitosan, low molecular weight chitosan (product number 448869) and high molecular weight chitosan (product number 419419) were purchased from Sigma-Aldrich (St. Louis, MO).

2.4. Molecular weight

The viscosity average molecular weight (M_v) was determined by using a TA Instruments (New Castle, DE) AR2000 rheometer. The chitosan samples were dissolved in 0.25 M acetic acid/0.25 M sodium acetate solution. The LW and HW samples were mixed using a stir bar overnight at room temperature, whereas the fungal chitosan sample was mixed using a stir bar for 48 h at room temperature. The sample was placed in a concentric cylinder and then