Biodegradable Chitosan Coating Gel from Agaricus Bisporus: A Natural Alternative to Chemical Preservatives

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Abstract

Chitosan is a degradable biopolymer with effective antimicrobial property, having a potential to be a promising alternative to chemical preservatives that cause health and environmental issues. Traditionally, chitosan is sourced from crustacean shells which lead to environmental problems such as unsustainable fishing practices, strong chemical usage, and marine pollution. This study extracted chitosan from *Agaricus bisporus* (champignon mushroom) which is an environmentally friendly alternative due to its rapid production, ability to grow on the agricultural waste, and less hazardous extraction methods. The mushroom derived chitosan was formulated into a edible coating gel by cross linking with glycerol. Fourier-transform infrared spectroscopy (FTIR) analysis revealed a degree of deacetylation of 79.09%, surpassing commercial chitosan from crustaceans at 72.19%. Scanning electron microscopy (SEM) of fruits coated chitosan gel shows a smooth surface, reflecting adhesion properties, and shelf-life evaluations on banana samples indicated that the application of the mushroom-based chitosan gel nearly doubled the preservation period. These results highlights the potential of mushroom derived chitosan as an alternative of chemical preservatives that can benefit health and environment, while also reduce the reliance of marine-derived sources.

Keywords: Mushroom-derived chitosan / Biodegradable edible coating / Food preservation / Marine-derived source / Environmental friendly alternatives

1. Introduction

The value of the global food preservatives market was 2.98 billion dollars in 2023, and is expected to be 4.65 billion dollars in 2030 [1]. These preservatives are widespread in versatile food products, from foods to snacks or drinks, due to the preferences for convenience and the industrial expansion [2]. Nevertheless, these chemical substances, such as benzoates and sorbates, which are widely known for notorious effects on health, including allergic reactions, endocrine distributions, microbiome imbalances, and proteinase inhibitions [3]. Surveillance reports from 2012 revealed that the amount of nitrate in food products were exceedingly

detected in Thailand, which can lead to formation of nitrosamines that are carcinogenic to the body. Consequently, consumers are at risk from gastric and esophageal cancer and other diseases such as asthma, ADHD, cardiovascular diseases, mental health disorders, and obesity [4][5][6]. Given the growing concerns over food safety and sustainable consumption, biomaterials become potential choices [7].

Chitosan is a biomaterial derived from saccharides that are components in several organisms, including crustaceans, insects, and mushrooms [8]. It has unique properties such as inert nature, hydrophilicity, biocompatibility, biodegradability, and non-toxicity [9]. Multiple studies confirmed its multifunctional benefits, including efficiency to increase antioxidant enzymes, prevent lipid oxidation, and inhibit microorganisms such as bacteria and yeast in food [10]. Additionally, chitosan can form durable and edible films which can be used for food packaging [11].

Traditionally, chitosan is extracted from crustaceans shells such as shrimp and crabs. However, this causes environmental concerns such as unsustainable fishing practices, strong chemical usage, generation of wastes, and marine pollution. Over 1.5 million hectares of coastal lowlands, including salt flat, marshes, and agricultural lands, have been converted into shrimp farms, significantly affecting biodiversity and marine ecosystem [12]. Moreover, crustaceans chitosan can be contaminated with proteins, making it challenging to be on food products for seafood-allergic consumers, particularly in Asia where approximately 7% of the population in several countries are seafood allergic [13][14].

Agaricus bisporus (Champignon mushrooms) is an edible mushroom belongs to the Basidiomycetes group and is responsible for 32% of the world's mushroom production [15]. It presents a promising alternative source of chitosan due to their rapid cultivation cycles, ability to grow on agricultural waste substrates, and flexibility to be produced worldwide [16][17].

Currently, most research focuses on the investigation of chitosan from crustaceans, and their application as films. [18] However, chitosan-based films have several limitations such as moisture sensitivity, poor elasticity, and difficulty to maintain in shapes with different food surfaces [19]. This study aims to extract chitosan from *Agaricus bisporus* and compare it with commercial chitosan from crustaceans source. The chitosan then further developed into a coating gel to investigate the effectiveness in extending shelf life and developing as an alternative of food preservatives to promote quality consumption and sustainable environment

2. Objectives

1. To extract and characterize chitosan from *Agaricus bisporus* as an alternative to crustaceans-derived chitosan
2. To develop a chitosan-based dipping gel for the assessment and effectiveness in extending shelf life
3. To compare the physical chemical properties between commercial and extracted chitosan

4. To assess sustainability and feasibility of *Agaricus bisporus* chitosan as an environmentally friendly food preservation solution

3. Scope of Study

This study focuses on the extraction of chitosan from *Agaricus bisporus* and its application in extending shelf life. The scope of the research includes the following:

1. Extraction and characterization of *Agaricus bisporus*-derived chitosan
2. Development of chitosan-based food preservation coating
3. Evaluation of characteristics and preservation efficiency
4. Promotion of sustainable and environmental impacts by reducing harmful preservatives, promoting sustainable agriculture, and improving health qualities.

4. Methodology

4.1 Material Preparation

750 grams of *Agaricus bisporus* were cut into smaller pieces, frozen at -80 degree Celsius for 16 hours, and lyophilized for 32 hours. The freeze-dried samples were stored at -40 degree Celsius for 48 hours. Moisture content was calculated by the initial weight (W1) and final weight after freeze-dry (W2), using the following formula:

$$\text{Moisture content (\%)} = 100 * (W1-W2)/W1 \quad (1)$$

4.2 Chitosan Extraction

Chitosan extraction followed the protocol from Abdelghani et al. (2020), with several adjustments. [16] 50 grams of samples were ground and soaked in 4% sodium hydroxide (NaOH) at a 1:30 (w/v) ratio on a hot plate for 2 hours to remove protein. The sample was then centrifuged, washed until the pH reached 7, and freezed at -80 Celsius for 48 hours before lyophilized again. To separate chitin, the sample was treated with 2% acetic acid (CH_3COOH) at 1:100 (w/v) ratio at 95 degree Celsius. The chitin was neutralized and deacetylated by using 60% NaOH at 1:50 (w/v) ratio for an hour at 95 degree Celsius to obtain chitosan. Chitin and chitosan yields were calculated by the following formulas:

$$\text{Chitin yield (\%)} = 100* (W3-W4)/W3 \quad (2)$$

$$\text{Chitosan yield (\%)} = 100 * (W4-W5)/W4 \quad (3)$$

(initial dry weight (W3), chitin weight (W4), and chitosan weight (W5))

4.3 Fourier transform Infrared Spectroscopy (FTIR) Analysis

The extracted chitin and chitosan, and commercial chitosan from shrimp (Sigma-Aldrich, USA) were analyzed using Tensor 27 FTIR (Bruker Optik GmbH, Germany). Spectra used were over 400-500 cm^{-1} of the range with a resolution of 4 cm^{-1}. The degree of acetylation (DA) of chitin and degree of deacetylation (DD) of chitosan were calculated using absorbance at ~1,655 cm^{-1} (amide I) and ~3,450 cm^{-1} (hydroxyl -OH) positions according to the equation:

$$DA\ (\%) = (A1655 - A3450) * 115 \quad (4)$$

$$DD(\%) = (100 - (A1655-A3450))*100 \quad (5)$$

4.4 Coating Gel Formation

The coating gel was prepared according to Sanchez-Gonzalez el at. (2010) with some modifications. [20] The gel is formed by mixing 1% (w/v) chitosan solution in 0.5% (v/v) acetic acid (v/v) at 40 degree Celsius for an hour. The plasticizer was added using 0.3% (v/v) glycerol and continued to stir at 40 degree Celsius for another hour. Then, the gel solution was poured into a petri dish and dried in an oven at 45 degrees Celsius for 2 hours.

4.5 Scanning Electron Microscope (SEM) Analysis

The surface morphologies of chitosan gel films and their adhesion on fruit peels were studied using Quanta 450 SEM (Fei, USA) with 5 to 15 kV tension, the samples were coated with a thin layer of gold to enhance the electrical conductivity. The pictures were captured with magnification of 400x and 100x.

4.6 Preservation Evaluation

Bananas from an identical comb were coated with chitosan gel and stored at 5°C for 24 hours before being transferred to a room temperature and observed for changes in color and texture compared to uncoated samples. The ripeness was evaluated using a standardized scale. (Figure 1)

Figure 1 standardized bananas ripeness scale [21]

5. Results and discussion

The moisture content of *Agaricus bisporus* was 91.2%. From an initial weight of 750 grams, the final weight after freeze-drying was reduced to 66 grams. This result aligns with other studies, where the moisture contents were examined with the average of 76.4±0.92 (Table 1) [23] [24]. The high moisture content can be due to the cell walls of *Agaricus bisporus* that are composed with hydrophilic chitin, glucans, and proteins that enhance the ability to absorb large amounts of water [25]. Additionally, its thin tissue structures increase the surface area to volume ratios, promoting water absorption and minimizing water loss [26].

The chitin yielded from *Agaricus bisporus* is 9.7% from its dry weight, while chitosan yielded is 4% of the extracted chitin. In comparison, traditional sources of crustacean-derived chitosan yield significantly higher chitin (averaging 27.5±12.5%) and chitosan (Table 1) (averaging 75±15% of the chitin weight) [27] [28] [29]. Theoretically, the difference is because of the exoskeleton of crustaceans shells that has chitin as the primary composition, whereas *Agaricus bisporus* has other additional components such as beta-glucans for their cell walls [30]. Nevertheless, chitin and chitosan yields are largely dependent on extraction methods and conditions. Some parts of the sample loss likely occurred during washing and neutralizing, making the measured weight slightly vary.

Table 1 Crustaceans shells extraction of the previous studies [27][28][29][36], compared to *Agaricus bisporus* extracted chitosan

Sources	Moisture Contents (%)	Chitin yield (%)	Chitosan yield (%)
Agaricus bisporus	91.2	9.7	4
Crustacean shells (shrimps)	76.4±0.92	27.5±12.5	75±15

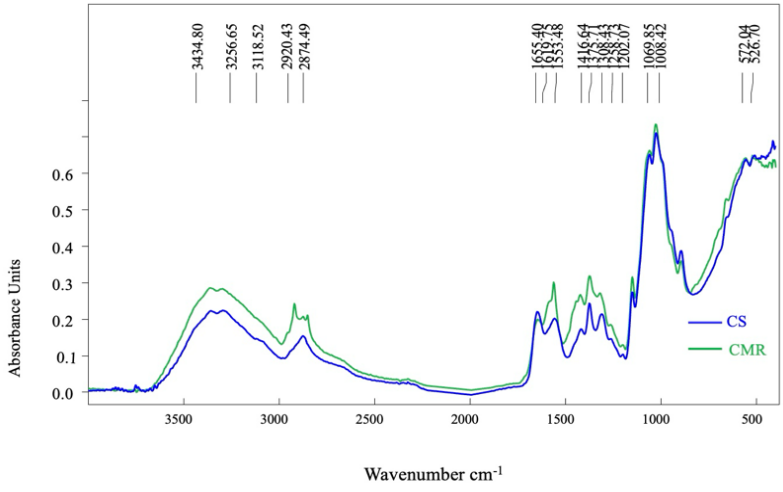

Figure 2 FTIR result (CS) Commercial Chitosan (CMR) *Agaricus bisporus* Chitosan

From Figure 2, both the commercial and extracted chitosan samples have similar shift on the 3,434-3,118 cm^{-1} range, indicating hydroxyl (-OH) and amine (-NH) groups that are typical for chitosan. There were additional peaks in 2,920-2,874 cm^{-1}, which is for C-H stretching that is common found in polysaccharides. The peak between 1,655 to 1,533 cm^{-1} suggest the presence of carbonyl (C=O) and residue acetyl groups. Based on the FTIR results, the composition of both samples are comparable, confirming purification of the extracted chitosan.

The FTIR results reported that the degree of deacetylation (DD) of *agaricus bisporus* chitosan was 79.09%, while commercial chitosan from shrimp was 72.20%. According to previous studies, moderate DD of shrimp-derived chitosan were also reported for around 70-75% [31]. The 6.89% higher DD in *Agaricus bisporus* chitosan shows a potential as an alternative chitosan source. Higher DD indicates a greater removal of acetyl groups, meaning they become more chitosan-like and have higher free amino groups. This enhances chitosan properties, including solubility, antimicrobial properties, biodegradability and film-forming ability [32] [33] [34] [35].

At 400x magnification (Figure 3), the uncoated sample of fruit peel showed roughness, porosity and natural wax. When there was the presence of coating gel, the surface became smoother with the reduction of scratching textures, indicating the gel's ability to improve surface uniformity. However, small residues were observed around the coated surface, suggesting that some part of chitosan might not completely dissolved and blended with the gel.

At 100x magnification (Figure 4), the uncoated sample has many scratches with a plain and thin surface. On the other hand, the coated sample exhibits a thick and smoother texture, though there were some inconsistencies in coverage. The observations point out chitosan gel's effective adhesion and protective capabilities, though some improvements in solubility and uniformity can be further improved.

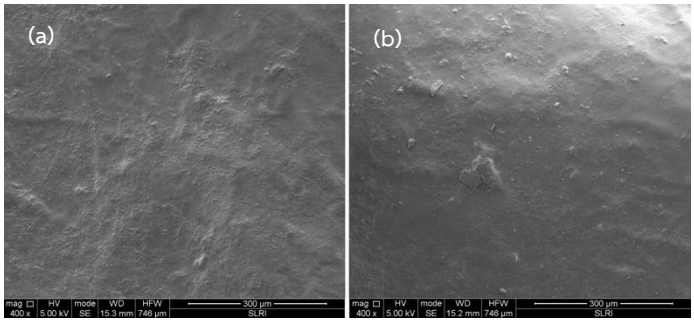

Figure 3 Surface Morphologies from 400X SEM (a) uncoated fruit sample (b) coated fruit sample

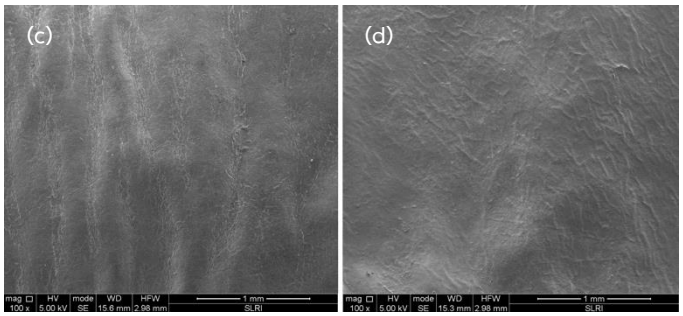

Figure 4 Surface Morphologies from 100X SEM (c) uncoated fruit sample (d) coated fruit sample

During the one-week evaluation (Table 2), uncoated bananas show a faster progression in ripening with significant changes in colors in day 5 and 7 that indicate browning. On the other hand, the coated sample exhibits a slower ripeness process with the fresh that still continues even after seven days.

By day 5, the uncoated banana showed a significant sign of ripening as they started to turn brown and soften. The coated one, however, remains firm and yellow.

By day 7 (Figure 5), the uncoated sample was over ripened and spoiled, whereas the coated one is still consumable. This is because the chitosan gel acts as a barrier and protection for the samples in order to avoid oxygen and ethylene gas which are two main factors of ripening. Moreover, due to its antimicrobial property, they effectively helped to delay the spoilage of the tested bananas.

Table 2 daily ripeness records in coated and uncoated bananas

Day	Ripeness scale of coated sample	Ripeness scale of uncoated sample
1	1	1
2	1	2
3	1	3
4	1	4
5	2	5
6	2	6 (blacken)
7	3	6 (blacken)

Day 1 Day 5 Day 7

Figure 5 Coated (left) and uncoated (right) banana sample

6. Conclusion

Despite its higher moisture content and lower chitin and chitosan yields, chitosan extracted from *Agaricus Bisporus* has a higher degree of deacetylation (79.09%) than commercial chitosan from crustaceans (72.19%). SEM results show smoother and thicker surfaces of the samples which highlight adhesion and protection properties, yet there was some residue that stuck with the gel and the surface, causing some uneven textures. The evaluation of the shelf life demonstrates the efficiency to extend the freshness of bananas, delaying ripening by up to four scales.

Future studies should focus on optimizing extraction methods to provide higher *Agaricus bisporus* chitosan yield, and increase solubility of the chitosan gel for more uniform coating. These findings highlight the potential of coating gels from chitosan, derived from *Agaricus bisporus* source as a sustainable alternative to conventional crustaceans-derived extraction and usage of chemical preservatives in food products.

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