

Antioxidant Properties and Functional Characteristics of Radiation-Processed Fungal Chitosan from *Ganoderma tsugae* Residue

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Abstract—The residue of edible and medicinal mushrooms contains many physiologically active substances, including glucosamine, N-acetyl glucosamine, chitin and chitosan, some of the compounds are the major ingredients of health food for the joints. Gamma radiation is known to cause main chain scissions in polysaccharides and molecular weight of the polymers. We determined the antioxidant property and antibacterial activity of chitosan and glucosamine prepared from the residue of *Ganoderma tsugae* by using γ -irradiation technology; in addition, the fat binding ability of chitosan was determined. Based on the results obtained, EC₅₀ values of chitosan and glucosamine hydrochloride were 0 and 7.32, 9.26 and 15.16, 2.30 and 2.63, 16.06 and 1.43mg/mL for antioxidant activity, reducing power, scavenging ability and chelating ability, respectively. Chitosan possessed 8.25–13.5mm antibacterial ability for *Salmonella typhimurium*, *Staphylococcus aureus*, *Bacillus cereus* and *Bacillus subtilis*. Glucosamine hydrochloride only inhibit the growth of *Listeria monocytogenes*. The fat binding ability of chitosan is 29.95g/g sample. Based on the results, chitin and glucosamine also have antibacterial and oil adsorption activity, as possible food supplements or ingredients or fur use in the pharmaceutical industry.

Index Terms—gamma irradiation, fungal chitosan, fungal glucosamine, *Ganoderma tsugae* residues, antioxidant properties, antibacterial activity, fat binding ability

I. INTRODUCTION

After extraction, the residue of edible and medicinal mushrooms contains many physiologically active substances, including glucosamine, N-acetyl glucosamine, glucuronic acid, chitin, chitosan and oligochitosan [1]–[3], and some of the compounds are the major ingredients of health food for the joints. In Taiwan, with advancing age, the requirement for health food for the joints is increasing. Currently, the use of mushroom residues is quite rare; they are mostly discarded or used as compost. Only a few

studies have used the traditional methods to extract oligochitosan, but a large amount of chemical reagent used causes an increase in preparation cost and environmental pollution [4], [5]. In the world, many researchers have used γ -rays to degrade polymer; however, the research on the application of γ -ray on mushroom residues is not available. Application of γ -irradiation could reduce the quantity of chemical reagent used and level of environment polluted; and the products would be edible for vegetarians and those allergic to shrimp and crab, as well as being used for development of health food for the joints [6], [7].

Because chitin and chitosan possesses many beneficial biological properties such as antimicrobial activity, biocompatibility, biodegradability, hemostatic activity, and wound healing property, much attention has been paid to their biomedical applications [8], [9]. Moreover, information on the preparation and properties of chitin and chitosan obtained from fungi, especially mushrooms residue, is not readily available. The objective of this study was to assess the antioxidant properties, antibacterial activity and fat binding ability of chitosan and glucosamine prepared from fungal chitin.

II. MATERIALS AND METHODS

A. Preparation of Chitosan

Residue of *G. tsugae* was obtained from the Yen-Ten Farm, Zhongpu, Chiayi County, Taiwan. *G. tsugae* residue was ground using a mill to obtain coarse power and treated with the method of Kurita *et al.* [10] with some modification. The *G. tsugae* residue powder was treated with aqueous sodium hydroxide solution at the ratio of 1:40 (w/v) at 100 °C for 3h to remove protein. The mixture was filtered with 200-mesh cloth and washed with deionized water to neutral. For the purpose of decolorization, the precipitate thus obtained was treated further with 1% potassium permanganate solution for 40min, and then reacted with 2.5% oxalic acid solution at

60 °C for 1h. Following decolorization, the precipitate was washed with deionized water to neutral. After freeze-drying, the product obtained was designated as purified GL-chitin. For the purpose of N-deacetylation, 1g of purified GL-chitin was treated with 30ml of 50% sodium hydroxide solution at 120 °C for 3h. After filtration with 300-mesh cloth, washing to neutral with deionized water and hot air drying, the product obtained was designated as purified GL-chitosan.

B. Preparation of Glucosamine with Various Dose γ -Irradiated

For the purpose of glucosamine, purified GL-chitosan was treated with 5.87 N HCl as irradiation media. A sample was packaged in polyethylene packaging. After packaging, the sample was transported to the China Biotech Corp., Taichung City, Taiwan. It was γ -irradiated with doses of 50 ($\pm 10\%$) kGy ^{60}Co (600000 Ci, $6.78 \times 10^2 \text{ kGy/h}$) at ambient temperature, and the product obtained was designated as purified GL-C3.

C. Determination of Antioxidant Properties

The antioxidant activity was determined by the conjugated diene method. [11] Reducing power was determined according to the method of Oyaizu. [12] Scavenging ability on 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich) radicals was determined on the basis of Shimada *et al.* [13] Chelating ability was determined according to the method of Dinis *et al.* [14] Effective concentration at 50% (EC_{50}) value (mg extract/mL) is the concentration at which the absorbance was 0.5 for reducing power; antioxidant activity and DPPH radicals were scavenged by 50%; and ferrous ions were chelated by 50%, respectively. EC_{50} value was obtained by interpolation from linear regression analysis.

D. Antibacterial Assay Using a Paper Disk Method

Samples were tested on six pathogenic bacteria using a zone of inhibition assay on PCA (E. coli, S. typhimurium, S. aureus, L. monocytogenes and B. subtilis). Bacterial number of a 16h culture in TSB was estimated by comparing the absorbance at 600nm, after dilution with TSB and calibration to a standard curve. [15] The concentration was then adjusted to 4 x 10⁸ Colony Forming Units (CFU)/mL using phosphate buffer (pH 7.21) and a 100 μL aliquot was evenly spread on agar plates using a glass rod spreader. Sterilized filter paper disks (Whatman No. 1 filter paper, 0.6cm in diameter) were arranged on the plate, and a 10 μL aliquot of test solutions was added to the paper disks. After the plates were incubated at 37 °C for 16h, the diameters of the distinctly clear zones were measured using a metric ruler. Four disks were used for each test compound at each concentration.

E. Fat Binding Ability

For determination of the fat-binding ability of chitosan, a modification of biopharmaceutical model developed by Czechowska-Biskup *et al.* [16] was applied, allowing for in vitro simulation of human digestive tract conditions. Chitosan solution (pH set with HCl to 2.0– such as the

natural stomach environment) and 3g of plant oil were placed in a 50cm³ Erlenmeyer flask and shaken at 300rpm for 2h at 37 °C. After that, 0.1 M NaOH solution was added to pH 7.0 (corresponding to the pH of duodenum fluid), and shaking was continued for 0.5h. The solution was cooled to room temperature and centrifuged (2500rpm, 30min). The layer of free (unbound) oil was carefully quantitatively removed. The remaining oil was weighed, and this mass was used to calculate the fat-binding capacity of chitosan. A blind sample, without chitosan, was always run in parallel. Binding Index: oil bound (g)/chitosan (g). The standard powder of chitosan was from sigma company; KG-chitosan was purchased from King Car group company.

F. Statistical Analysis

All the measurements were in triplicate. The experimental data were subjected to an analysis of variance for a completely random design to determine the Fisher least significant difference among means at $P < 0.05$.

III. RESULTS AND DISCUSSION

A. Antioxidant Properties

The degree of deacetylation value of GL-chitosan was 67.27%. The antioxidant properties assayed herein are summarized in Table I, and the results were normalized by computing the effective concentration of chitosan and glucosamine samples at which the effect was 50% or the absorbance was 0.5 and expressed as EC_{50} values (mg production per ml) for comparison. Effectiveness in antioxidant properties was inversely correlated with EC_{50} value. For the antioxidant activity, the EC_{50} values of GL-C3 were 7.32mg/mL, indicating that was excellent antioxidant activity for free radicals. With regard to reducing power, the EC_{50} values of GL-chitosan (9.26mg/mL) were lower than those of GL-C3 (15.16mg/mL). For the scavenging ability on DPPH radicals, there was no difference in the EC_{50} values between GL-chitosan and GL-C3 samples. For chelating ability on ferrous ions, the EC_{50} values of GL-C3 were 1.43mg/mL, indicating that they were excellent chelators for ferrous ions. Overall, GL-chitosan was effective in reducing power and scavenging ability on DPPH radicals; GL-C3 was effective in antioxidant activity and chelating ability on ferrous ions, respectively.

TABLE I. EC_{50} VALUE OF GL-CHITOSAN AND GL-C3 IN ANTIOXIDANT PROPERTIES

	EC_{50} (mg production yied/mL)	
	GL-chitosan	GL-C3
Antioxidant activity	-	7.32 \pm 0.02 ^a
Reducing power	9.26 \pm 0.17	15.16 \pm 1.12
Scavenging ability	2.30 \pm 0.39	2.63 \pm 1.28
Chelating ability	16.06 \pm 1.20	1.43 \pm 0.02

^a Each value is expressed as mean \pm standard deviation ($n=3$).

B. Antibacterial Activity

The two test compounds showed various degrees of inhibition against the six bacterial strains using the paper disk assay (Table II). The inhibition was not dose-related,

and the difference in zone size between the test concentrations was not significantly different. GL-chitosan showed not dose-related increase in zone of inhibition against *S. typhimurium*, *S. aureus*, and *B. subtilis*, while GL-chitosan showed a similar effect only

against *B. cereus*. GL-C3 showed a zone of inhibition (8.00-10.25 cm) against *L. monocytogenes*; however, the inhibition was not dose-related. Therefore, these compounds could serve as potential antibacterial agents to inhibit pathogen growth in food.

TABLE II. ANTIBACTERIA ACTIVITY OF GL-CHITOSAN AND GL-C3

Sample	Concentration (mg/mL)	<i>E.coli</i>	<i>S. typhimurium</i>	<i>S. aureus</i>	<i>L. monocytogenes</i>	<i>B. subtilis</i>	<i>B. cereus</i>
GL-chitosan	Blank	- ^a	-	-	-	-	-
	20	-	13.00±1.41A ^b	8.25±0.35A	-	9.25±2.74A	9.00±1.14A
	40	-	13.50±2.12A	11.00±2.83A	-	9.00±0.01A	8.50±0.01A
	60	-	12.75±0.35A	10.50±0.71A	-	9.00±0.01A	-
	80	-	10.50±0.71A	11.25±0.35A	-	9.00±1.41A	-
	100	-	11.00±0.01A	10.50±2.12A	-	10.50±0.71A	-
GL-C3	Blank	-	-	-	-	-	-
	0.5	-	-	-	-	-	-
	1	-	-	-	8.00±0.01A	-	-
	5	-	-	-	10.25±0.35A	-	-
	10	-	-	-	10.00±0.01A	-	-
	20	-	-	-	8.50±2.12A	-	-

^a “-” means no inhibition zone diameter. ^b Each value is expressed as mean ± SD (n=3). Means with different letters within a row are significantly different (p<0.05).

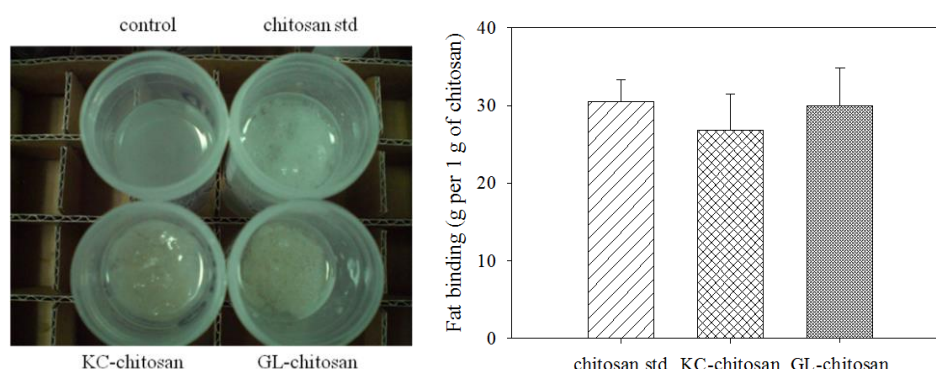


Figure 1. Adsorbent activity of GL-chitosan.

C. Adsorbent Activity of GL-Chitosan in Oil

Our tests using the biopharmaceutical model of the digestive tract show that chitosan binds appreciable amounts of edible oil. The results for the fat binding activity of the chitosan samples ranged from 26.79-30.47g per 1g of chitosan (Fig. 1). Czechowska-Biskup [16] reported that the average fat binding activity of commercial crab chitosan for rape-seed oil was 20g/g, respectively. The values obtained were higher than the values reported by Czechowska-Biskup. [16] Fat binding activity signifies how the chitosan can easily bind or absorb fat, especially when used in the manufacturing of dietary supplements.

IV. CONCLUSIONS

The chitosan and glucosamine prepared from the residue of *Ganoderma tsugae* by suitable medium with γ -irradiation can produce more physiologically active substances. The results showed fungal chitosan was good in antioxidant properties, especially scavenging ability on DPPH radicals and chelating ability on ferrous ions. In addition, the fungal glucosamine has more effective antioxidant properties, except for reducing power. The

ability to inhibit bacteria, chitosan on *S. typhimurium* has the best inhibitory effect, up to 13.5mm zone of inhibition. Glucosamine hydrochloride has the best inhibitory ability to *L. monocytogenes*, inhibition zone up to 10.25mm. In addition, oil adsorption activity of chitosan and the control group was between 26.79-30.47g/g sample; there was no significant difference. On the basis of the results obtained, the fungal chitosan and glucosamine with alleged antioxidant properties may be used as an antioxidant source. Chitin and glucosamine also have antibacterial and oil adsorption activity, as possible food supplements or ingredients or for use in the pharmaceutical industry.

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