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Comparison of Chitosan Extraction Processes from Oyster Mushrooms (pleurotus pulmonarius): Chemical versus Green Extraction Methods

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Abstract: ***Chitosan is a natural substance derived from chitin. It can be extracted from oyster mushrooms (pleurotus pulmonarius) and is biodegradable. Its benefits include slowing down the spoilage of fresh food and it is safe for humans. Objective: To study and compare the results of chitosan extraction from oyster mushrooms using chemical and green extraction methods. Furthermore, to develop alternative production processes that are safe and environmentally friendly. Method: The green method substituted conventional chemicals with ethanol, fruit-derived acetic acid, and ash lye solutions for defatting, demineralization, deproteinization, and deacetylation. Chitosan yield and FTIR functional group characteristics were evaluated. Results: The green extraction method produced a higher chitosan yield (40.78 g; 4.078% w/w) than the chemical method (10.04 g; 1.06% w/w). FTIR analysis showed comparable absorption peaks associated with chitosan functional groups in both products. Conclusions: These findings suggest that the green extraction method may provide an environmentally safer and more efficient alternative for chitosan production from oyster mushrooms.***

Keywords: chitosan, sustainable extraction, green chemistry, oyster mushroom, FTIR analysis, biomaterials

1. Introduction

Chitosan is a natural substance derived from the processing of chitin. It possesses cationic polymer properties, enabling it to bind with negatively charged substances such as fats and certain heavy metals. It is also soluble in acidic solutions such as acetic acid, and exhibits multiple beneficial properties including fat and toxin absorption, as well as inhibition of the growth of various microorganisms, bacteria, and fungi. Chitosan has a wide range of applications: in agriculture, it helps extend the shelf life of fresh food[1], and promotes plant growth while protecting against plant diseases in the food industry[2], it serves as a food preservative and fat-absorption agent in healthcare[3], it is used for wound healing and infection prevention[4,5], as well as in the development of pharmaceuticals and medical materials such as sutures and components of drug-controlled release systems; and in cosmetics, it is used as an ingredient in skin and hair care products due to its moisture-retention properties[6]. In terms of environmental applications, chitosan helps prevent water pollution by absorbing toxins and heavy metals from wastewater,[7,8] and is suitable for the production of biodegradable materials such as bioplastic bags, as it is biodegradable, non-allergenic, and considered safe for public use.[9] As consumers today place greater emphasis on health consciousness and show an increasing tendency to avoid synthetic preservatives, the development of safe and effective natural preservatives such as chitosan represents a significant and promising option for broad application in the food industry.[10] Chitosan is therefore of considerable interest for study and promotion as a contributor to the food industry economy.

Chitosan can be extracted from low-cost food waste raw materials found in hard-shelled and segmented-legged animals, such as the shells of shrimp, crabs, and insects, as well as from fungal cell walls.[9] It can additionally be extracted from certain edible mushroom species, including shiitake, oyster, Caesar's mushroom, abalone, brown ear, and straw. The chitosan content in these mushrooms, ranked from highest to lowest, is 2.25%, 1.54%, 1.26%, 1.12%, 0.50%, 0.47%, and 0.45%, respectively.[11,12] Considering the top four mushrooms with comparable chitosan content, oyster mushrooms are the most interesting candidate, as they are easy to cultivate, readily available, sold year-round, affordable, and increasingly popular for consumption.[13] The research team therefore believes that studying or experimenting with chitosan extracted from oyster mushrooms should be convenient and yield results comparable to those obtained from food waste raw materials.

Oyster mushrooms are higher fungi that develop into fruiting bodies or clusters visible to the naked eye. The fruiting bodies range in color from white to light brown, with firm, darker-colored caps and white stems that are long and lack a surrounding ring. Oyster mushrooms are highly nutritious, being rich in protein and vitamins, low in fat, and high in fine dietary fiber.[14] A key advantage of using oyster mushrooms for chitosan extraction experiments is that they are easier to source and store compared to animal shells, which require careful temperature-controlled storage to prevent spoilage. Furthermore, chitosan extracted from oyster mushrooms and applied as a fresh food preservative offers greater benefits in reducing the risk of food allergies compared to chitosan derived from hard-shelled and segmented-legged animals such as shrimp.[15] Accordingly, chitosan extraction from

oyster mushrooms represents another viable alternative with a conveniently accessible raw material source.

The chitosan extraction process begins with chitin-containing raw materials. Based on a review of the literature, the process can be summarized in several steps: 1) Raw material preparation, which involves cleaning oyster mushrooms with clean water to remove fat and protein impurities, followed by drying through methods such as oven drying or sun drying to conserve energy; 2) Deproteinization and demineralization, in which chitin is extracted using chemical solutions- sodium hydroxide (NaOH) to break down and remove proteins, and hydrochloric acid (HCl) to eliminate calcium carbonate minerals; 3) Chitosan extraction from chitin via deacetylation, using concentrated sodium hydroxide at high temperatures of approximately 80–100°C to remove acetyl groups (-$COCH_3$) from the chitin structure, ultimately yielding chitosan; and 4) Purification, in which the chitosan is washed with clean water to remove residual chemicals and then dried under appropriately controlled temperature conditions.[16-18]

In light of the above, green chitosan extraction processes should be promoted as alternatives to chemical methods- for example, using ethanol instead of petroleum ether for defatting oyster mushrooms, fruit-derived acetic acid instead of hydrochloric acid for demineralization, and ash lye solution instead of sodium hydroxide for both deproteinization and deacetylation. To ensure efficient chitosan extraction, the percentage yield should be clearly measured. Also, this includes comparing the actual yield with the theoretical yield using Fourier Transform Infrared Spectroscopy (FTIR) to examine the absorption values within the wavenumber range for functional group analysis of chitosan.[19] The resulting product is pure chitosan, which is likely to be a safer alternative for consumer use.

2. Objectives

- To investigate the green and consumer-safe extraction of chitosan from oyster mushrooms.
- To compare the chitosan yield obtained from oyster mushrooms through chemical versus green extraction methods.

3. Methods

3.1 Specific and Operational Definitions

1) Chitosan refers to a natural substance found in hard-shelled and segmented-legged animals or fungi. When calcium, proteins, and unwanted minerals are extracted and removed, the resulting substance has a chemical structure similar to cellulose and is known as chitin.
2) Green extraction refers to experimental procedures conducted with primary consideration for environmental sustainability.
3) Deionized Water (DI) is purified water free of ions (electrical charges), having undergone a filtration process that removes mineral ions such as calcium, magnesium, sodium, and chloride, resulting in a highly pure water.
4) Ash lye solution is water obtained by soaking or fermenting wood ash in plain water, which causes certain minerals to dissolve and renders the water highly alkaline.
5) Natural acetic acid (natural vinegar) is an organic acid found in nature and produced through biological fermentation processes.
6) Glycerol tween 80 is a yellow liquid used as a food additive of the emulsifier type. Tween 80 is the trade name for the chemical compound known as Polysorbate 80.
7) Deacetylation is a chemical process used to remove acetyl groups (-$COCH_3$) from a molecule, resulting in the formation of other functional groups such as amine groups (-NH_2) in their place. This process typically employs concentrated alkalis (NaOH, KOH) or enzymes (Deacetylase Enzyme).
8) Defatting is the process of removing fats and oils from raw materials in order to prevent interference with subsequent extraction steps.
9) Acid demineralization is the process of removing minerals such as calcium carbonate from raw materials in order to obtain pure chitin.
10) Deproteinization is the process of removing proteins associated with chitin in raw materials in order to obtain pure chitin.
11) Percentage yield (%yield) is a value used to measure the efficiency of a chemical process or experiment by comparing the actual yield obtained with the theoretical yield expected, expressed as a percentage.
12) FTIR is a technique that uses infrared light to identify types of chemical bonds or functional groups within molecules, based on the principle of analyzing molecular vibrations upon exposure to infrared light energy. When a molecule's specific frequency generates a peak at a particular wavenumber position, an infrared spectrum fingerprint corresponding to the molecular bonds of that substance is produced. When a substance is measured using an FTIR instrument, a spectrum graph is obtained showing the positions of various functional groups such as O–H, N–H, C=O, and C–H.[19] The FTIR technique can therefore be applied to examine absorption values within the wavenumber range used for identifying functional groups in chitosan.

3.2 Materials, Equipment, and Chemicals

1) Equipment consists of: 1) beakers, 2) magnetic stirrer, 3) pH meter, 4) hot air oven, 5) two-decimal-place analytical balance, 6) water bath, 7) graduated cylinder, 8) filter screen, 9) reagent bottles, and 10) FTIR (Fourier Transform Infrared Spectrometer).
2) Raw material: 1,000 g of oyster mushrooms, cut into small pieces and sun-dried.
3) Chemicals consist of: 1) 95% ethanol, 2) 2% (w/v) HCl, 3) 2% (w/v) NaOH, 4) 50% (v/v) sodium hydroxide (NaOH) solution, 5) glycerol, 6) Tween 80, 7) 3% (w/v) acetic acid (vinegar), and 8) ash lye solution at pH 13.

3.3 Experimental Procedure

1) Chemical Preparation

The chemical preparation steps are as follows: 1) 2% (w/v) sodium hydroxide (NaOH) solution- weigh 2.00 g of NaOH into a beaker, dissolve in a small amount of DI water, stir with

a glass rod until fully dissolved, transfer to a 100 mL volumetric flask, and adjust the volume to 100 mL with DI water to obtain a 2% (w/v) NaOH solution; 2) 50% (w/v) sodium hydroxide (NaOH) solution- weigh 2.00 g of NaOH into a beaker, dissolve in a small amount of distilled water, stir until dissolved, transfer to a 100 mL volumetric flask, and adjust the volume to 100 mL with DI water to obtain a 50% (w/v) NaOH solution; 3) 2% (w/w) hydrochloric acid (HCl)- prepared from a 37% (w/w) HCl stock solution by measuring 5.40 mL into a 100 mL volumetric flask (acid added to water), then adjusting the volume to 100 mL with DI water to obtain a 2% (v/v) HCl solution; and 4) 2% (w/w) acetic acid (CH_3OOH)- diluted from a 5% (w/v) acetic acid solution by measuring 40.0 mL into a 100 mL volumetric flask and adjusting the volume to 100 mL with DI water to obtain a 2% (w/w) solution.

2) Chemical extraction

The chemical extraction process consists of the following steps: 1) Raw material preparation- approximately 1 kilogram of fresh oyster mushrooms is weighed, shredded into fine strips, spread evenly on a baking tray, and dried in an oven at 50°C for 4.5 hours; 2) Defatting- the dried mushrooms are soaked in 95% ethanol (ratio of 1 gram per 5 mL, total volume 550 mL) for 2 minutes, then removed and placed on a rack to dry, in order to remove oils and impurities; 3) Demineralization- the prepared mushrooms are boiled in 2% (w/w) hydrochloric acid (HCl) at a ratio of 1 gram of mushroom per 8.77 mL of acid at 60°C for 2 hours, washed with DI water 3 times (pH measured: first wash pH 6, final wash pH 4), filtered, and left to dry for 1 day, then further dried at 30°C to obtain a clean solid; 4) Deproteinization — the solid is boiled with 2% (w/v) NaOH (ratio of 1 g per 8 mL) at 60°C for 2 hours, washed with DI water 3 times, dried at 50°C for 1 hour, then re-dried at 75–100°C for approximately 30 minutes to 1 hour; and 5) Chitosan production (Deacetylation) — the chitin obtained from the previous step is soaked in 50% (w/v) NaOH at 90°C for 7 hours (with gentle stirring every 2 hours), washed with DI water 3 times, filtered until pH is neutral, then dried again at 50°C. The substance obtained at this stage is chitosan, ready for further use.

3) Green extraction

The green extraction process consists of the following steps: 1) Raw material preparation- approximately 1 kg of fresh oyster mushrooms is weighed, shredded into fine strips, spread evenly on a tray, and sun-dried until fully dry; 2) Defatting- the dried mushrooms are soaked in 95% ethanol (ratio of 1 g per 5 mL, total volume 550 mL) for 2 minutes, then removed and placed on a rack to dry, in order to remove oils and impurities; 3) Demineralization- the prepared mushrooms are boiled in 2% (w/w) pineapple-derived acetic acid (CH_3OOH) at a ratio of 1 g of mushroom per 8.77 mL of acid at 60°C for 2 hours, washed with DI water 3 times (pH measured: first wash pH 6, final wash pH 4), filtered, and sun-dried until fully dry to obtain a clean solid; 4) Deproteinization- the solid is boiled with ash lye solution at pH 12.80 (ratio of 1 g per 8 mL) at 60°C for 2 hours, washed with DI water 3 times, and sun-dried until fully dry; and 5) Chitosan production (Deacetylation)- the chitin obtained from the previous step is soaked in ash lye solution at pH 12.85 (ratio of 1 g per 8 mL) at 90°C for 7 hours (with gentle stirring every 2 hours), washed with DI water 3 times, filtered until pH is neutral, then sun-dried until fully dry. The substance obtained at this stage is chitosan, ready for further use.

3.4 Functional Group Analysis Using FTIR Spectrophotometer

Functional group analysis using the FTIR Spectrophotometer followed the method consisted of the following steps:[20] 1) grind the chitosan standard into a fine powder; 2) mix the chitosan standard with potassium bromide (KBr), an inorganic compound appearing as white, odorless crystals with a slightly salty taste, then grind the mixture in a mortar until uniformly distributed; 3) place the ground chitosan standard into a mold and press using a hydraulic press, leaving it for approximately 15 minutes; 4) remove the mold clamp- the chitosan standard will remain in the mold as a transparent disc, with the chitosan standard dispersed on the KBr pellet, ready to be loaded into the FTIR instrument; and 5) repeat the experiment using chitosan extracted from oyster mushrooms via both the chemical and eco-friendly processes, following steps 1–4 identically each time.

4. Results

4.1 Chitosan extraction results

A comparative study of chitosan extraction between the chemical and green processes revealed the following. Step 1, the mushrooms were shredded into small pieces at 1,000 g and dried: the chemical process used oven drying, yielding 114.46 g of dried mushroom, while the green process used sun drying (with constant monitoring to maintain a consistent temperature), yielding 114.10 g. Step 2, the remaining mushroom from each process was then subjected, defatting with ethanol, leaving 114.06 g and 78.30 g, respectively. Step 3, demineralization with hydrochloric acid produced clean solids of 100.05 g and 66.39 g, respectively. In Step 4, deproteinization of the solids using 2% sodium hydroxide yielded chitin of 56.48 grams and 62.00 g, respectively. Final step, deacetylation using 50% sodium hydroxide produced chitosan of 10.04 g (%yield = 1.06) and 40.78 g (%yield = 4.08), respectively, as shown in Figure 1.

(i) Chemical extraction

(ii) Green extraction

Figure 1: Chitosan extraction results

A comparison of chitosan extraction rates from equal quantities of oyster mushrooms showed that green extraction methods yielded a higher percentage yield than chemical extraction methods. Details are shown in Table 1.

Table 1: Comparison of chitosan extraction results using chemical and green extraction methods

Processes	Period	Chemical extraction			Green extraction		
		Trial (g)		%yield	Trial (g)		%yield
		before	after		before	after	
1. Prepare and dry the mushrooms.	4.5 hours	1,000	114.46	11.45	1,000	114.10	11.41
2. Remove grease stains (ethanol)	2 minutes	114.46	114.06	11.41	114.10	78.30	7.86
3. Demineralization (HCl 2%)	2 hours	114.06	100.05	10.01	78.30	66.39	6.64
4. Extract the solid from the protein (NaOH 2%).	2 hours	100.05	56.48	5.94	66.39	62.00	6.20
5. Acetyl group removal (NaOH 50%)	7 hours	56.48	10.04	1.06	62.00	40.78	4.08

4.2 Results of Functional Group Analysis Using FTIR Spectrophotometer

Functional group analysis of chitosan using the FTIR technique is based on the principle that chitosan is a derivative of chitin, and chitin contains an acetamide group (NH-CO-CH_3) with a C=O bond of the amide molecule, exhibiting absorption in the wavenumber range of 1,870–1,650 cm^{-1}. Upon removal of the acetyl group, the acetamide group of chitin is converted to the amino group (-NH_2) of chitosan, with an -N-H (Bending) bond of the amine molecule, which exhibits absorption in the wavenumber range of 1,640–1,550 cm^{-1}.[20, 21] The functional groups of the chitosan standard were examined using the FTIR Spectrophotometer technique, and the results are presented as a graph in Figure 2.

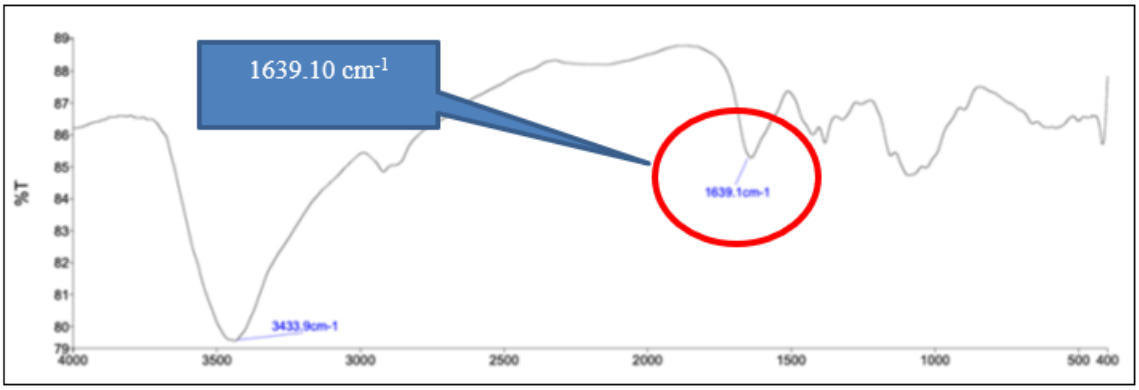

Figure 2: Standardized functional groups of chitosan

4.3 Chitosan extraction by chemical process

Functional group examination using FTIR Spectrophotometer revealed that the spectrogram of chitosan from chemical extraction occurred at the –N-H (bending) functional group, with an absorption value at a wavenumber of 1637.23 cm^{-1}. The results are shown in the graph in Figure 3.

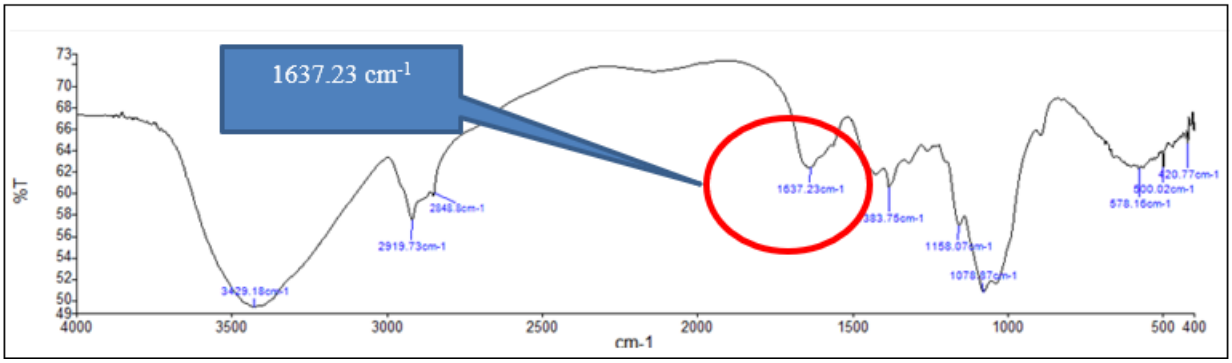

Figure 3: Chemical extraction functional groups of chitosan

4.4 Chitosan extraction by green process

Functional group examination using FTIR Spectrophotometer revealed that the spectrogram of chitosan extracted using the green method occurred at the –N-H (Bending) functional group, with an absorption value at wavenumber 1645.41 cm^{-1}. The results are shown in the graph in Figure 4.

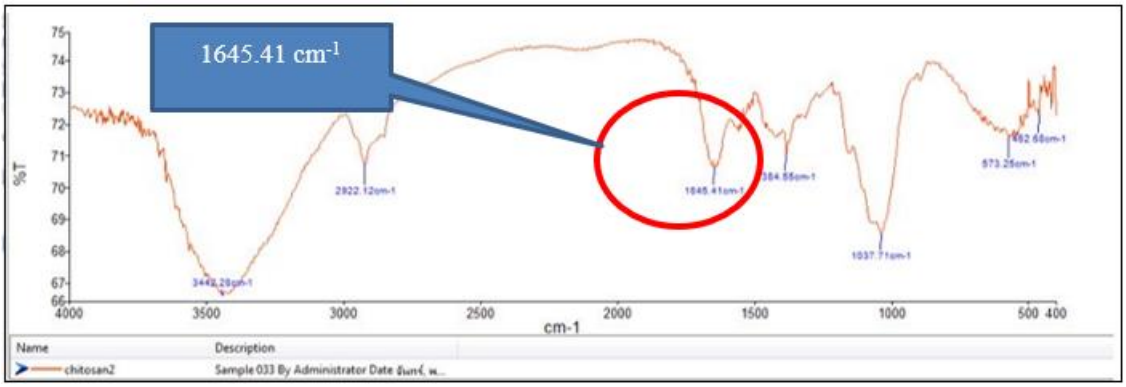

Figure 4: Green extraction functional groups of chitosan

Both chemical and green extraction methods yielded chitosan. Functional group analysis using FTIR spectrophotometer revealed that both methods exhibited chitosan spectrograms at the -N-H (bending) functional group with absorption values of 1637.23 cm^{-1} and 1645.41 cm^{-1}, respectively. These values are close to the standard values used for chitosan functional group analysis.

5. Discussion

The experiment on chitosan extraction from oyster mushrooms using both chemical and green extraction processes was conducted as follows. The chemical extraction method consisted of: 1) defatting with 95% (w/w) ethanol, 2) demineralization with 2% (w/w) HCl, 3) deproteinization with 2% (w/v) NaOH, and 4) deacetylation with 50% (w/v) NaOH. Starting from 1,000 g of oyster mushrooms, the process yielded 10.04 g of pure chitosan extract at a %yield of 1.06%. The green extraction method consisted of: 1) defatting with 95% (w/w) ethanol, 2) demineralization with 2% (w/v) acetic acid (pineapple-derived vinegar), 3) deproteinization with ash lye solution (pH 12.80), and 4) deacetylation with ash lye solution (pH 12.85). Starting from 1,000 g of oyster mushrooms, the process yielded 40.78 g of pure chitosan extract at a %yield of 4.08%. Functional group analysis using the FTIR Spectrophotometer revealed that the chitosan spectrogram exhibited an -N-H (Bending),[22] functional group with absorption peaks at wavenumbers of 1,637.23 cm^{-1} for the chemical extraction process and 1,645.41 cm^{-1} for the green extraction process. Both extracts can be confirmed as chitosan, as their absorption wavenumber values fall within the standard reference range of 1,640–1,550 cm^{-1}, which is lower than the absorption wavenumber range of chitin at 1,870–1,650 cm^{-1}.[21]

Regarding the green extraction process of chitosan, the drying step employed sun drying instead of electrical oven drying. Sun drying requires careful control of both duration and temperature through continuous monitoring of weather conditions to maintain heat levels comparable to microwave oven drying. This limitation may introduce some experimental error. However, the advantage lies in the development of an energy-saving green extraction process, which would be beneficial for commercial chitosan production.

6. Conclusion and Recommendations

Chitosan extraction using the green process- involving sequential steps of sun drying, defatting, demineralization, deproteinization, and deacetylation of oyster mushrooms- yields pure chitosan. The green extraction method produced a higher chitosan yield than the conventional chemical method while showing comparable FTIR functional group characteristics. These findings indicate that green extraction may be a promising environmentally friendlier alternative for chitosan production, although further optimization and statistical validation are recommended.

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