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CHITIN
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Properties of chitin extracted from Estonian mushrooms

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Abstract. Fungi contain a significant amount of chitin in their cell walls presenting an attractive source for this commercially significant material. In this study, chitin was extracted from eight different mushroom species native to Estonia. Significant differences in molar mass, chitin content, and fibre size were observed while the degree of acetylation was mostly similar.

Key words: chitin, fungi, mushroom, viscosity.

1. INTRODUCTION

Chitin is everywhere. It is in the food we eat [1], cosmetics we wear [2], and increasingly in advanced medical applications [3]. It is also the second most abundant naturally occurring polymer after cellulose, found in insects, shellfish, and fungi. Even though chitin can be found in many different organisms, the vast majority of commercial chitin is derived from shrimp and crab waste. While this source material is ideal for many applications due to its extreme abundance and low price, the presence of parasitic organisms commonly found on shrimps and crabs can cause issues for some industries [4,5].

Researchers have shown that a base and acid extraction process commonly used for crab and shrimp can produce quality chitin from a diverse array of source materials including honeybees [6], mushrooms [7], and crawfish [8] among others.

Mushrooms have several advantages compared to traditional source materials primarily the lack of crustacean parasites and the ability to cultivate them in a wide variety of climates. While studies have shown that chitin can be extracted from mushrooms [5], a comprehensive comparison among different species is missing. Most of the previous studies have shown that the properties of chitin extracted from different source materials can vary noticeably [8], making this kind of study important to identify potential future sources.

The aim of this study was to characterize the properties of chitin extracted from wild mushrooms chosen primarily for their natural abundance. Determining the differences, or similarities, among mushroom species could lead to new commercial sources for chitin production as well as a deeper understanding of basic fungi biology.

2. MATERIALS AND METHODS

2.1. Source materials

Eight species of mushroom (detailed in Table 1) were collected from forested areas around southwestern Tallinn. The samples were rinsed with water to remove

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Table 1. Reaction details and fibre dimensions with standard deviation (SD)

Mushroom	Yield	Degree of acetylation (SD)	Fibre dimensions, nm		
			Length (SD)	Width (SD)	Aspect ratio (SD)
Amanita muscaria	13.1%	58.9% (3.0%)	189.6 (14.3)	12.2 (2.2)	15.9 (2.3)
Amanita pantherina	10.9%	57.2% (10.2%)	296.4 (211.9)	15 (3.9)	18.3 (9.6)
Cantharellus cibarius	7.9%	46.4% (3.4%)	195.3 (35.2)	9.0 (3.4)	23.5 (6.9)
Fomes fomentarius	43.1%	52.5% (2.8%)	292.9 (107.1)	13.2 (2.7)	23.7 (11.4)
Pholiota gummosa	16.4%	52.8% (2.7%)	392.0 (133.7)	13.4 (6.8)	34.8 (17.3)
Russula nigricans	18.5%	57.7% (2.2%)	154.4 (24.1)	10.8 (0.6)	14.3 (2.5)
Russula vinosa	28.2%	76.1% (2.9%)	1748.9 (1906.6)	18.5 (7.6)	97.1 (119.4)
Tricholoma terreum	16.8%	33.2% (2.1%)	153.7 (27.0)	10.4 (1.6)	15.2 (4.3)

foreign material and dried for 24 h at 70 °C. Finally, the particle size was reduced with a coffee grinder and stored in sealed containers until needed.

2.2. Extraction

Chitin was extracted from the mushroom powder by a modified base-acid extraction. Approximately 40 g of mushroom powder was added to 200 mL of 2 M sodium hydroxide at reflux for 1 h to remove other organic material. The samples were then centrifuged with deionized (DI) water until neutral (approx. 10 times). Once neutral, the wet samples were mixed with 200 mL of 2M hydrochloric acid at room temperature to remove any inorganic components. Once again, the samples were centrifuged with DI water until neutral. Finally, the wet samples were added to 200 mL of a 2% (by weight) solution of sodium hypochlorite (bleach) for 5 min. It is critical that the product is washed quickly, a longer reaction time will degrade the chitin. Dry chitin is extremely difficult to solubilize, therefore after centrifuging the sample back to neutral, it was stored in a solution of DI water (approximately 5–10% by weight).

2.3. Degree of acetylation determination

A small film (radius approx. 1 cm) was cast from the chitin solution to be tested via infrared spectroscopy on a Nicolet IR100 FT-IR or Interspec 200X. In both cases, attenuated total reflection (ATR) was used to obtain a spectra of the chitin film. A carbonyl group is present in chitin (DA = 100%) but not chitosan (DA = 0%). Comparing the intensity of the absorbance peak at 1655 cm^{-1} from the carbonyl group to the hydroxyl peak at 3450 cm^{-1} was used to determine the degree of acetylation of the different chitin samples using the following equation [9,10]:

$$DA\% = \frac{A_{1655}}{A_{3450}} \times \frac{1}{1.33} \times 100\%.$$

2.4. Viscosity average molar mass

The molecular mass of chitin was determined by finding the intrinsic viscosity using an Ubbelohde capillary viscometer. 1 mg/mL solutions of chitin in 0.1 M acetic acid and 0.2 M sodium chloride at 25 °C were used. The relative viscosity (η_r) and specific viscosity (η_{sp}) were determined comparing the time required for the solvent to transverse the viscometer to the time required for a dilute chitin solution. The intrinsic viscosity was then determined using the equation developed by Solomon and Ciuta [11,12] with c being the concentration

$$\eta = \frac{[2(\eta_{sp} - \ln \eta_r)]^{1/2}}{c}.$$

This viscosity was used to determine the molar mass using the Mark-Houwink-Sakurada equation

$$\eta = K(M_v)^{\alpha},$$

where $K = 1.81 \times 10^{-3}$ mL/g and $\alpha = 0.93$ which are dependent on the solvent-polymer system [13]. This solvent and using the Solomon and Ciuta approach to determine the viscosity have been shown to correspond closely to known values and have small variation making it the preferred choice for chitin molar mass determination [12].

2.5. Fibre size

Transmission electron microscopy (TEM) was performed with a Tecnai Spirit Bio TWIN at the Electron Microscopy Center at Indiana University-Purdue University Indianapolis to measure the length and width of individual chitin fibres. The instrument was run at 80 kV with images obtained at magnifications from 80,000 to 120,000.

3. RESULTS AND DISCUSSION

The yield of the extraction reactions, approximately equivalent to the amount of chitin in the source material, varied considerably among the mushrooms studied. The highest amount of chitin by far was found in the only polypore investigated in this study, *Fomes fomentarius*. The original sample had a mass greater than 1 kg and lost very little moisture during the drying process.

The degree of acetylation (DA) was mostly consistent for the chitin samples with only one, *Russula vinosa*, exceeding 60%. These values are lower than chitin extracted from other source materials under similar conditions [14]. This discrepancy does not correspond to the yield nor the size of the fibres. One significant difference between fungi and other insect/crustacean sources is the location of the chitin. In fungi, the chitin is found in the cell walls while the shells of crustacean and exoskeletons of insects have a mostly uniform distribution. The mushroom samples appeared disperse into solution quicker than previously studied insect and crustacean materials, which could lead to effectively longer reaction times.

Most of the chitin studied showed little variation in the dimensions of the fibres when dispersed in a 2 M acetic acid solution before casting. However, *Russula vinosa* had small fibres similar to other source materials (seen in the background of Fig. 1) as well as fibres with a very high aspect ratio. With higher magnification, these fibres do not appear to be aggregates but rather single-walled fibres. The morphology of chitin has been shown to vary based on the region of the mushroom it was extracted from [15], which could explain this discrepancy.

The viscosity average molar mass (Fig. 2) was significantly lower (approximately 500,000 g/mol) than chitin extracted from other sources [14] including other fungi [16]. Taken with the lower DA, this implies that the reaction conditions were harsher than necessary resulting in some amount of degradation.

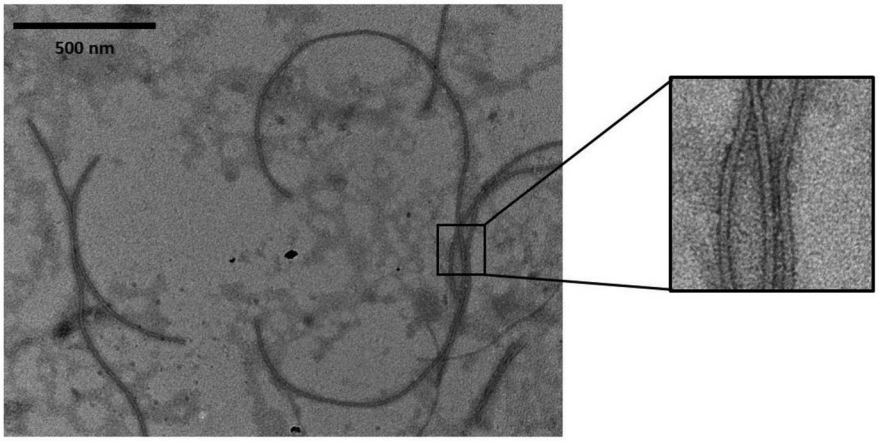

Fig. 1. TEM image of chitin fibres extracted from *Amantia muscaria*.

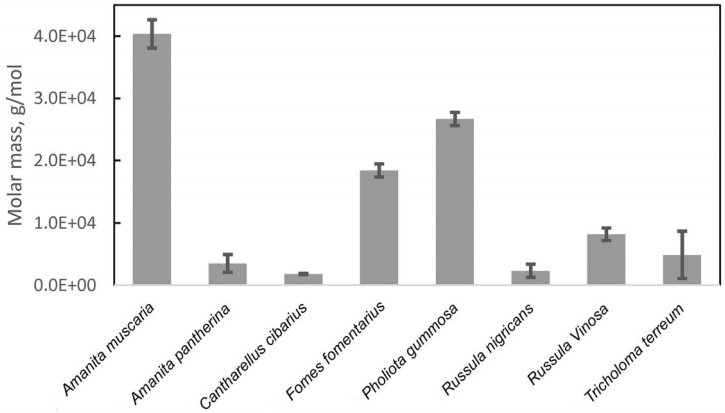

Fig. 2. Viscosity average molar mass of extracted chitin.

4. CONCLUSIONS

Chitin was successfully extracted from eight mushroom different mushroom samples. The resulting nanofibres had aspect ratios from 26.1–96.1, which is slightly higher than average chitin nanofibres. The average yield and DA were similar to other source materials while the molar mass was lower. These results show that any and all of these mushrooms can produce chitin suitable for a wide variety of applications.

One major issue with the commercialization of chitin from mushrooms is the low chitin content. Most mushroom species contain a large amount of water making the yields reported in Table 1 appear deceptively high. However, the low moisture content and large size of *Fomes fomentarius* combined with a very high chitin content negates these typical drawbacks. *Fomes fomentarius* also had the highest DA and one of the highest molar masses. While the other species studied here grew in the course of days, *Fomes fomentarius*, along with many other polypores, does take many years to reach a large size.

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Eesti seentest ekstraheeritud kitiini omadused

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