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Chitin Nanopaper from Mushroom Extract: Natural Composite of Nanofibers and Glucan from a Single Biobased Source

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ABSTRACT: An isolation method with mild mechanical agitation and no acidic extraction step from a mushroom substrate resulted in chitin nanofibers (ChNFs) with large shares of retained glucans (50–65%). The subsequent chitin nanopapers exhibited exceptionally high tensile strengths of >200 MPa and moduli of ca. 7 GPa, which were largely attributed to the preserved glucans in the mixture, imparting a composite nature to the nanopapers. The isolation method for ChNFs is notably different from the conventional process with crustacean chitin sources that do not incorporate glucans and where an acidic extraction step for the removal of minerals must always be included.

KEYWORDS: Alternative biomass, Chitin nanofibers, Mechanical properties, Mild extraction, Nanopaper, Nonwoven networks

INTRODUCTION

Nanosized filaments such as carbon nanotubes, metal oxide nanowires, and polymer nanofibers are intensively researched within contemporary materials science. In this area, nanofibers based on renewable resources has been an emerging topic for the past decade. Aside from protein-based alternatives like amyloid and silk, native polysaccharides, e.g., cellulose and chitin have been frequently employed. Polymer nanofibers in general have commonly been manufactured via bottom-up electrospinning from dissolved state, but the native supramolecular ordering of polysaccharides as nanosized fibrils enables a more robust top-down route for their isolation, usually set in aqueous environment. In the case of cellulose and chitin, the top-down isolated nanofibers possess unusually high mechanical properties because of their structural role in nature. Therefore, it has been popular to envision the use of polysaccharide nanofibers as reinforcing components in sustainable composites or on their own in nonwoven, often transparent or translucent networks called nanopapers. This Letter shows how chitin nanofibers (ChNFs) can be isolated using a simple preparation method (Figure 1), which is optimized for the materials performance for the subsequent nanopapers. Special attention is paid to mild, acid-free isolation with minimal energy consumption.

Crustaceans are the most common and well-known source for chitin and chitin nanofibers that are generally isolated via demineralization, deproteinization, and subsequent mechanical disintegration. Although a number of new isolation techniques for ChNFs from diverse sources, including mushrooms, have emerged, there is still room for improvement concerning the optimization of properties for particular target applications. Here, we demonstrate how a mild preparation method based almost entirely on hot water, alkaline extraction, and low-energy blending could utilize mushrooms to produce ChNFs while preserving glucans within the ChNF matrix. The benefit of maintaining the amorphous glucan was demonstrated in preparations of nanopapers whose tensile properties exceeded those of other reported ChNF nanopapers.

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RESULTS AND DISCUSSION

Figure 1 shows the scheme for the preparation of ChNF from the common mushroom Agaricus bisporus. After 5 min of low-tech blending to increase the surface area, the A. bisporus tissue was then subjected to 30 min hot water extraction at 85 °C to remove the directly water-soluble components, followed by alkaline deproteinization in 1 M aqueous NaOH for 3 h at 65 °C. Figure 1a and 1b illustrate the appearance of the remaining fraction after extraction and neutralization. When the fraction was diluted to 0.8% w/v concentration, a fluid dispersion emerged (Figure 1c). It was essential not to dry the mushrooms after the extraction steps as a drying step leads to unstable dispersions; only the never-dried sample yielded a stable dispersion after a 7 day trial (Figure 1d).

Electron micrographs in Figure 2 show that the samples consist of ChNFs. As the control reference, we applied a common crustacean chitin source from Cancer pagurus which was subjected to exactly the same treatment as the mushroom source with an additional and necessary demineralization step in 1 M HCl for 30 min prior to the alkaline extraction. Furthermore, a 10 min postblending step was required to separate the nanofibre bundles in the C. pagurus extract (Figure 2a vs 2b) whereas no postblending was required for the mushroom extracts (Figures 2c–d vs 2e–f). Furthermore, we chose to separately treat the mushroom stalk and cap to see whether the location of chitin had any effect on the resulting ChNFs. Expectedly, the crustacean source amounted to conspicuous ChNFs, which were further separated by a post blending step (Figures 2a and 2b). All mushroom samples, be it stalk, cap or both combined, led also to ChNFs (Figures 2c–f), although they were not as distinct in the mushroom extract as they were when isolated from the crustacean source. The somewhat blurred appearance of mushroom ChNFs is due to a sizable content of amorphous glucan as shown in the monosaccharide composition after a total hydrolysis of the polysaccharides for analysis (Table 1).

The carbohydrate analysis in Table 1 showed that the ratio of chitin (represented by glucosamine) and glucans (represented by glucose and other sugars) was ca. 50/50 in the mushroom cap and ca. 35/65 in the mushroom stalk. These values are similar to those reported for A. bisporus elsewhere.21 In terms of C. pagurus, the crustacean exoskeletons lack glucans to begin with and also mannans were largely removed when demineralization by acid had to be performed to obtain the crustacean ChNFs, leading to a far lower content of other sugars than glucosamine. Although the chitin content was lower in the mushroom extracts, their overall yields were superior: 25.4% from the stalk and 15.0% from the cap, with respect to just 9.7% from the crustacean source.

Although the remaining glucan decreases the proportion of ChNFs in the mushroom extracts compared with the

Figure 1. Flowchart of nanofiber extraction from the common cultivated Agaricus bisporus: (a) Extract from 3 kg whole mushroom containing approximately 42 g nanofibers, (b) Consistency of 3% w/v extract obtained after chemical extraction, (c) 0.8% w/v whole mushroom suspension dispersed by 1 min postblending; this never dried suspension was used for nanopapers. (d) Stability test of 0.8% w/v whole mushroom chitin suspension after 7 d; left = never dried suspension; middle = resuspension of freeze-dried sample (fast freezing using liquid nitrogen); right = resuspension of freeze-dried sample (slow freezing using common freezer).

Figure 2. SEM images of air-dried 0.01% w/v suspension of: (a) C. pagurus extracts that were not subjected to any postblending. Note that the fibers are still in their aggregate form, (b) C. pagurus extracts after being subjected to additional 10 min postblending. (c,d) Whole mushroom extracts that were not subjected to any postblending. (e) Stalk extract after 1 min postblending, (f) Cap extract after 1 min postblending.
crustacean extracts (Figure 2, Table 1), the resulting nanopapers—prepared by simple filtration of the ChNF dispersion—were significantly tougher and stronger than the corresponding nanopapers from the crustacean ChNFs in the tensile test (Figure 3, Table 2). In fact, the tensile strength for

of 190 MPa and 6 GPa modulus but the high values required the use of additional chitosan in the process. All these reports utilized a crustacean source for ChNFs, thus not including native glucans intrinsic in our mushroom source. We hypothesize that it is precisely the presence of glucan which causes the enhanced binding between the ChNFs in our nanopapers, thereby improving both tensile strength and modulus. Located between the semi-crystalline chitin fibril scaffold in the matrix, glucans impart a composite character to the nanopapers and they may also act as favorable cross-linkers between the ChNFs—just like they do in the plant cell wall between cellulose microfibrils. Glucans are predominantly amorphous, which showed up as decreased crystallinity in the X-ray diffractograms (Figure S1, Table S2). Because of the amorphous glucan, however, the surface area and porosity of the mushroom extracts was decisively low (Table 2), also contributing to the increased strength and toughness. We must note that the grammage of the nanopapers had to be optimized to 80 g/m² in order to achieve the optimal strength for the nanopapers (Table S4).

Figure 4a shows the appearance of the nanopapers. The nanopapers from mushroom-based ChNFs are more translucent than those based on C. pagurus (Figure 4b). The brown color in ChNFs most possibly originates from the melanins in the spores and gills (the spore bearing structure underneath the mushroom cap). Consequently, the nanopapers from cap

Table 1. Nanoﬁber Extract YIELD per original dry weight, moisture content, and sugar analysis of the freeze-dried sample on a dry weight basis

<table>
<thead>
<tr>
<th>Sample</th>
<th>Extract yield (%)</th>
<th>Moisture content (%)</th>
<th>Total sugar (%)</th>
<th>Glucosamine (%)</th>
<th>Glucose (%)</th>
<th>Mannose (%)</th>
<th>Xylose (%)</th>
<th>Galactose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. pagurus</td>
<td>9.7</td>
<td>6.2</td>
<td>69.0</td>
<td>58.3</td>
<td>0.0</td>
<td>10.7</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>A. bisporus Stalk</td>
<td>25.4</td>
<td>7.7</td>
<td>83.6</td>
<td>29.8</td>
<td>46.8</td>
<td>5.8</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>A. bisporus Cap</td>
<td>15.0</td>
<td>7.6</td>
<td>79.7</td>
<td>32.3</td>
<td>39.6</td>
<td>6.5</td>
<td>0.9</td>
<td>0.4</td>
</tr>
<tr>
<td>A. bisporus Whole mushroom</td>
<td>15.0</td>
<td>7.6</td>
<td>79.7</td>
<td>32.3</td>
<td>39.6</td>
<td>6.5</td>
<td>0.9</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Table 2. Density (ρ), Porosity (P), and Tensile Properties of Investigated Chitin-Based Nanopapers

<table>
<thead>
<tr>
<th>Sample</th>
<th>ρ (g/cm³)</th>
<th>P (%)</th>
<th>Tensile strength (MPa)</th>
<th>Young modulus (GPa)</th>
<th>Elongation at break (%)</th>
<th>Tensile toughness (MJ/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. pagurus</td>
<td>1.40</td>
<td>67.3</td>
<td>69.5 ± 4.6</td>
<td>2.7 ± 0.5</td>
<td>6.2 ± 0.7</td>
<td>2.7 ± 0.4</td>
</tr>
<tr>
<td>A. bisporus Stalk</td>
<td>1.50</td>
<td>65.0</td>
<td>191.9 ± 10.6</td>
<td>5.0 ± 0.3</td>
<td>9.2 ± 1.3</td>
<td>10.1 ± 1.6</td>
</tr>
<tr>
<td>A. bisporus Cap</td>
<td>1.47</td>
<td>62.3</td>
<td>192.9 ± 12.0</td>
<td>5.2 ± 0.5</td>
<td>7.4 ± 1.5</td>
<td>8.0 ± 1.6</td>
</tr>
<tr>
<td>A. bisporus Whole mushroom</td>
<td>1.47</td>
<td>59.0</td>
<td>204.4 ± 4.0</td>
<td>6.9 ± 1.2</td>
<td>5.3 ± 0.4</td>
<td>5.3 ± 0.5</td>
</tr>
</tbody>
</table>
ChNFs were darker than those from stalk ChNFs. Possible methods to remove the color, including a phenol-alcohol treatment, will be systematically researched in a future study. Because of the increased toughness, the mushroom-based nanopapers were altogether relatively resilient and, unlike the crustacean-based nanopapers, could be folded like standard paper (Figure 4c). In terms of thermal stability (Figure S2, Table S3), on the other hand, ChNFs from C. pagurus had a slightly higher degradation onset temperature at 280 °C than the mushroom-based ChNFs at 230–240 °C. This may be due to the presence of glucans that allegedly start degrading at lower temperatures than, for example, glucose-free mannans which were the detected polysaccharides in crustacean-based ChNFs (Table 1). Similarly, the distinctions in the glucan content may help explain the differences in water uptake (Figure S3, Table S3): the distinctively higher amount of amorphous glucans in the mushroom-based ChNFs (Table 1) results in an increased water uptake at 90%RH compared with that of ChNFs from C. pagurus. All in all, slightly reduced thermal stability or marginally increased water uptake capabilities are not quantitatively significant to distinguish the materials properties of mushroom-based ChNFs from an engineering perspective.

The performance properties and straightforward production pathway of this mushroom chitin nanopaper suggest very good prospects for a favorable sustainability profile for its manufacture and use. At present, we know that annual production of mushrooms and truffles (18.1 million tons) exceeds that of crustaceans (12.6 million tons), as reported by FAOstat data for 2017.27 However, a number of factors influence the quantitative assessment of sustainability. A clear next step in the ongoing research and development work is a rigorous, quantitative evaluation of the sustainability of its sourcing, processing, manufacture, use and disposal/recycling (e.g., using Life Cycle Assessment approaches). Nevertheless, based on the overall greener route, we can safely assume at the moment that this method for fungal ChNF production the greenhouse gas emission will be significantly less than 55 kg CO2-eq (per kg of chitosan) which is the estimated figure for crustacean chitin production in Europe.28

**CONCLUSIONS**

This study has demonstrated that mushroom tissue is an exceptionally viable alternative for the acquisition of ChNFs. Crustaceans have been by far the dominant source of chitin and ChNFs to date. The scarce accounts describing chitin or ChNF isolation from mushrooms to date have employed methods similar to those with crustacean materials, i.e., harsh mechanical treatments combined with acid extraction. High shear in grinding may induce mecanochemical degradation of chitin. Furthermore, the majority of acid-labile glucans are hydrolyzed and removed by the acid. Our novel results indicate that glucans are essential for the higher mechanical properties of the eventual ChNF nanopaper. They are not only the highest values hitherto reported for any ChNF nanopapers but they also approach the high values reported for cellulose nanopapers.11−13

**ASSOCIATED CONTENT**

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.9b00721.

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