## **Electronic Supplementary Information**

## Comparative Analysis of Chitin Isolation Techniques from Mushrooms: Toward Sustainable Production of High-Purity Biopolymer

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Figure S1. Process flow of pulping method with NaOH.



igure S2. Process flow of extraction using the [C<sub>2</sub>mim][OAc] microwave method.











Mushroom Biomass (White)



Wh/Chitin<sub>NaOH-24</sub>



Wh/Chitin<sub>[C2mim][OAc]</sub> Thermal



Wh/Chitin<sub>[C4mim][HSO4]</sub> Figure S5. Appearance of biomass and isolates.



Wh/Chitin<sub>NaOH-2</sub>



Wh/Chitin<sub>[C2mim]</sub>[OAc] Microwave



Wh/Chitin<sub>DES</sub>



**Figure S6.** FTIR spectra (4000 – 600 cm<sup>-1</sup>) of biomass (red), commercial chitin (pink), commercial chitosan (grey), and isolated materials by four different methods: pulping with NaOH–2 h (purple), pulping with NaOH–24 h (dark green), extraction with [C<sub>2</sub>mim][OAc]–microwave (cyan), extraction with [C<sub>2</sub>mim][OAc]–thermal (olive line), pulping with [C<sub>4</sub>mim][HSO<sub>4</sub>] (light green), and pulping with 2:1 Lactic Acid:[Cho]Cl (blue).



**Figure S7.** FTIR spectra (expanded in the region of  $1200 - 850 \text{ cm}^{-1}$ ) of biomass (red), commercial chitin (pink), commercial chitosan (grey), and isolated materials by four different methods: pulping with NaOH–2 h (purple), pulping with NaOH–24 h (dark green), extraction with [C<sub>2</sub>mim][OAc]–microwave (cyan), extraction with [C<sub>2</sub>mim][OAc]–thermal (olive line), pulping with [C<sub>4</sub>mim][HSO<sub>4</sub>] (light green), and pulping with 2:1 Lactic Acid:[Cho]Cl (blue).

Source	Type of Glucans	<sup>13</sup> C chemical shift (δ <sub>C</sub> , ppm)						D C
		C-1	C-2	C-3	C-4	C-5	C-6	Ket
Pleurotus ostreatus	β-glucan	103.3–103.6	74.1–74.4	86.6-85.4		76.0	68.9–69.1 & 62.0– 62.3ª	1
Pleurotus ostreatus	α-1,3-glucan	100.9–101.0	71.5	84.5-82.2			60.5	1
Pleurotus ostreatus	$(1 \rightarrow 3, 1 \rightarrow 6)$ - $\beta$ -D-glucan	103.6	65–79	86.1 <sup>b</sup>	80	65–79	62.4 and 69.3 for (1 $\rightarrow$ 3)- and (1 $\rightarrow$ 6)- linkage, respectively	2
Pleurotus tuber-regium (Fr.) Sing	β-D-glucan	102.6	72.8	85.9	68.1	75.6	69.4, & 60.3ª	3
Penicillium chrysogenum	(1→3)-α-D- glucan	101.2	71.95	83.66	71.46	73.63	62.03	4
Aspergillus fumigatus	$(1\rightarrow 3)$ - $\alpha$ -glucan	101.0	71.9	84.6	69.5	71.7	60.5	5
Aspergillus fumigatus	$(1\rightarrow 3)$ - $\beta$ -glucan	103.6	74.4	86.4	68.7	77.1	61.2	5
Aspergillus niger	(1→3)-α-D- glucan	100.0	72.7	73.9	79.8	71.4	60.9	6
Aspergillus niger	$(1\rightarrow 4)$ - $\alpha$ -D- glucan	101.0	71.4	83.2	70.3	73.5	61.3	6
Flammulina velutipes	Linear (1→4)-α- D-glucan	102.8	74.0	76.1	79.8	73.2	63.4	7
Flammulina velutipes	Branched $(1 \rightarrow 4, 1 \rightarrow 6)$ - $\alpha$ -D-glucan	102.4	74.2	76.1	79.8	73.2	72.2	7
Termitomyces eurhizus	$(1 \rightarrow 3)$ - $\beta$ -D-glucan	103.3	73.9	86.3	68.7	76.2	61.1	8
Agaricus bitorquis	$(1\rightarrow 6)$ - $\beta$ -D-glucan	103.4	73.5	76.0	69.9	75.3	69.2	9
Ganoderma lucidum	Branched $(1 \rightarrow 3, 1 \rightarrow 6)$ - $\beta$ -D-glucan	103.0	73.0	85.8	68.7	75.0	68.7	10

Table S1. Chemical Shift in the <sup>13</sup>C CP-MAS NMR spectrum of various mushroom glucans.

<sup>a</sup> referring to *O*-substituted units and unsubstituted units, respectively. <sup>b</sup> corresponding to  $(1\rightarrow 3)$ -linked residues of C-3



Figure S8. Solid-state <sup>13</sup>C CP-MAS NMR spectrum of crustacean chitin (extracted with [C<sub>2</sub>mim][OAc]).



Figure S9. Solid-state <sup>13</sup>C CP-MAS NMR spectrum of White Mushroom biomass.



Figure S10. Solid-state <sup>13</sup>C CP-MAS NMR spectrum of Wh/Chitin<sub>NaOH-24.</sub>



Figure S11. Solid-state <sup>13</sup>C CP-MAS NMR spectrum of Wh/Chitin<sub>[C2mim][OAc] Thermal</sub>.



Figure S12. Solid-state <sup>13</sup>C CP-MAS NMR spectrum of Wh/Chitin<sub>[C2mim][OAc] Microwave</sub>.



Figure S13. Solid-state <sup>13</sup>C CP-MAS NMR spectrum of Wh/Chitin<sub>[C4mim][HSO4]</sub>.



Figure S14. Solid-state <sup>13</sup>C CP-MAS NMR spectrum of Wh/Chitin<sub>DES</sub>.



**Figure S15**. 1D <sup>13</sup>C–Cross Polarization NMR spectrum of rigid polysaccharides of *A*. *fumigatus* cell walls. Abbreviations are used for resonance assignments. For example, A1 denotes  $\alpha$ -1,3-glucan carbon 1. Ch and B represent chitin and  $\beta$ -1,3-glucan, respectively. Adapted from Ref [11].



**Figure S16**. TGA weight loss (%) thermograms of isolated materials by different methods: pulping with NaOH–2 h (purple), pulping with NaOH–24 h (dark green), extraction with  $[C_2mim][OAc]$ –microwave (cyan), extraction with  $[C_2mim][OAc]$ –thermal (olive line), pulping with  $[C_4mim][HSO_4]$  (light green), and pulping with 2:1 Lactic Acid:[Cho]Cl (blue).



Temperature (°C)

**Figure S17**. DTG<sub>max</sub> thermograms of isolated materials by four different methods: (purple) pulping with NaOH–2 h, (dark green) pulping with NaOH–24 h, (cyan) extraction with [C<sub>2</sub>mim][OAc]–microwave, (olive line) extraction with [C<sub>2</sub>mim][OAc]– thermal, (light green) pulping with [C<sub>4</sub>mim][HSO<sub>4</sub>], and (blue) pulping with 2:1 Lactic Acid:[Cho]Cl.



**Figure S18**. PXRD diffractograms of of biomass (red) and isolated materials by four different methods: pulping with NaOH–2 h(purple), pulping with NaOH–24 h (dark green), extraction with  $[C_2mim][OAc]$ –microwave (cyan), extraction with  $[C_2mim][OAc]$ –thermal (olive line), pulping with  $[C_4mim][HSO_4]$  (light green), and pulping with 2:1 Lactic Acid:[Cho]Cl (blue).



Figure S19. SEM images (x 250 magnification) of the surface of chitin fiber obtained from Wh/Chitin $_{\rm NaOH-24}$ 



Figure S20. SEM images (x 500 magnification) of the surface of chitin fiber obtained from Wh/Chitin<sub>[C2mim][OAc]</sub>



**Figure S21**. Stress-strain curves of chitin fibers prepared from chitin (dark green) pulped from mushroom biomass with NaOH–24 h, (olive) extracted from mushroom biomass with  $[C_2mim][OAc]$ -thermal, (pink) pulped from crustacean biomass with  $[C_2mim][OAc]$ .

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