Preparation of Chitin Nano Whiskers from Mushrooms

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Abstract: Mushroom has been valued throughout the world as a source of both food and medicine for thousands of years. The objective of this study has focused on the edible mushrooms (*Pleurotus ostreatus* and *Agaricus bisporus*) preparation of chitin nanowhiskers from mushrooms. Chitin nanowhiskers were prepared from both the mushrooms by acid hydrolysis method after the removal of contaminating proteins and other minerals. The concentration of acid for hydrolysis was standardized for the two mushrooms. Button mushroom has yielded maximum chitin (14.3%) when 9N HCl was used and the oyster mushroom showed maximum chitin yield (44.45%) when 12N HCl was used for hydrolysis. Thickness, length and surface characteristics of the chitin were assessed through SEM. Size of the nanowhiskers of the mushrooms ranged from 50-200nm in length and ~11nm of thickness.

Key words: Mushrooms, acid hydrolysis, chitin nanowhiskers, SEM

1. INTRODUCTION

Chitin is a natural, renewable, biodegradable and most abundant natural polymer after cellulose [1]. Chitin occurs mainly in the exoskeleton of arthropods, the internal flexible backbone of cephalopods, worms, webs of spiders, cell walls of fungi and yeasts [2]-[4]. Chitin is considered to be a major biomass resource [5], with an annual production of more than 1011 tons in nature [6]. Chitin is non-toxic, odorless, and biocompatible with living tissues [2], presenting antibacterial, moisture retaining and healing characteristics [7]. Chitin can be utilized in water purification [8], as additives in cosmetics [9]-[10], as antibacterial agent [11]-[12], as a pharmaceutical adjuvant[11]-[13], in the production of paper, textile finishes, production of photographic products, cements, heavy metal chelating agents, membranes, hollow fibers, and for the removal of unwanted materials [14]-[16]. In addition to all these chitin has extensive biomedical applications such as tissue engineering scaffolds, wound dressings, separation membranes, antibacterial coatings, stent coatings, and sensors[13],[15]-[18], since it is harmless to the human body [19]. Dietary fibers of chitin are useful for improving functional foods.

In nature chitin exists as nanowhiskers in the exoskeletons of arthropods, embedded in matrices of proteins and minerals. The nanowhiskers have a uniform width of 10–20 nm and a long fiber length. The mushrooms are macrofungi with a fleshy and spore-bearing fruiting body. They contain chitin in their cell walls acting as a structural component. Though much research has been

done on chitin from arthropods and their applications in the various field, chitin from mushroom is less explored. Therefore, our study has been focused on chitin from the mushroom.

2. MATERIALS AND METHODS

All chemicals used in this study were of AR grade and purchased from Himedia Laboratories (Mumbai, India). Two edible species of mushrooms, *Agaricus bisporus* (White button mushroom) and *Pleurotus ostreatus* (Indian oyster mushroom) were purchased from the local market and used for the study.

2.1. Preparation of chitin nanowhiskers

Chitin nanowhiskers were prepared by subjecting the chopped fresh mushroom to acid hydrolysis [20]. Oyster and button mushrooms were suspended in a 5 % aqueous KOH solution (w/v) and boiled for 6 h under stirring in order to remove contaminating proteins. The dispersion was rinsed with distilled water and filtered. The resulting paste was kept at room temperature overnight under agitation. Subsequently, the boiling, washing and filtering steps were repeated. The chitin fibers obtained were bleached with 17% solution of $NaOCl_2$ in 0.3M Sodium acetate buffer of pH 4.0 heated at 80ºC for 2h with stirring. This procedure was repeated three times with rinsing. The resulting dispersion was centrifuged for 15 min at 1500rpm. Chitin was hydrolyzed with HCl by boiling for 90 min with stirring and the product was washed thoroughly with distilled water, followed by centrifugation (for 15 min at 5000rpm) and decanting the supernatant. This process was repeated three times.
The suspensions of chitin nanowhiskers were transferred to a dialysis bag and dialyzed for 2 h in running tap water and, then, kept overnight in distilled water. The product was further subjected to a supplementary dialysis for 12 h, changing the distilled water every 2 h; the dialysis was performed until a pH 6 was reached. The dispersion of nanowhiskers was accomplished further by three successive 2 min ultrasonic treatments. The dispersions were, subsequently, filtered to remove residual chitin nanocrystal aggregates. This is followed by the addition of appropriate volumes of 0.1M HCl solution until a pH of about 2.5 was reached. The particles were concentrated by dialysis against poly-(ethylene glycol). The solid fraction of this aqueous suspension was separated and kept at 6ºC in a refrigerator until used after adding chloroform to avoid development of microorganisms.

1.1. Characterization of chitin nano whiskers

Fourier-Transform Infrared Spectroscopy Analysis

Infrared spectra of KBr and chitin mixtures were obtained over the frequency range of 400 to 4,000 cm−1 at a resolution of 4 cm−1 using FTIR Spectrometer, Shimadzu , IR affinity 1S. The sample was thoroughly mixed with KBr, and the dried mixture was then pressed to result in a homogeneous sample disk.

In order to prepare the pellet 1.5mg of the sample was mixed with 350mg of KBr. Ground mixture well and 100mg of the powder was spread uniformly in the die and pressed to make the pellet The baseline was recorded and the pellet was mounted on the pellet on the die holder of the spectrophotometer with the help of tweezer. The spectrum was recorded from 400 to 4,000 cm−. The background (baseline) reading was subtracted and the resulting values were compared with a spectrum of standard chitin.

SEM

Length and surface characteristics of the chitin nanowhiskers were determined through SEM. This is carried out at the Centre for Nano Science and Engineering (CNSE), IISc, Bangalore with an SEM, ZEISS, Germany, model ULTRA55. Keep sample on gold coating plate coated at 120seconds, on electron beam, of diameter 100nm, the signal used for coating secondary electron.

3. RESULTS

4. COMPARISON OF CHITIN YIELD BETWEEN TWO MUSHROOMS.

The yield of chitin from button mushroom varied between 0.25% to 14. 3% and the highest yield of 14.3% was recorded at a concentration of 9N HCl. The oyster mushroom has yielded 30.1% to 44.45 % chitin under different concentrations of HCl, with the highest percentage under 12N HCl. The button mushroom has recorded an average of 32.22% more chitin content than button mushroom (Table 1).

FTIR

Results of the FTIR spectroscopic analysis of chitin are presented in Figure 1 to Figure 3. Infrared absorption spectrum of the sample is concordant with the spectrum of the chitin standard.

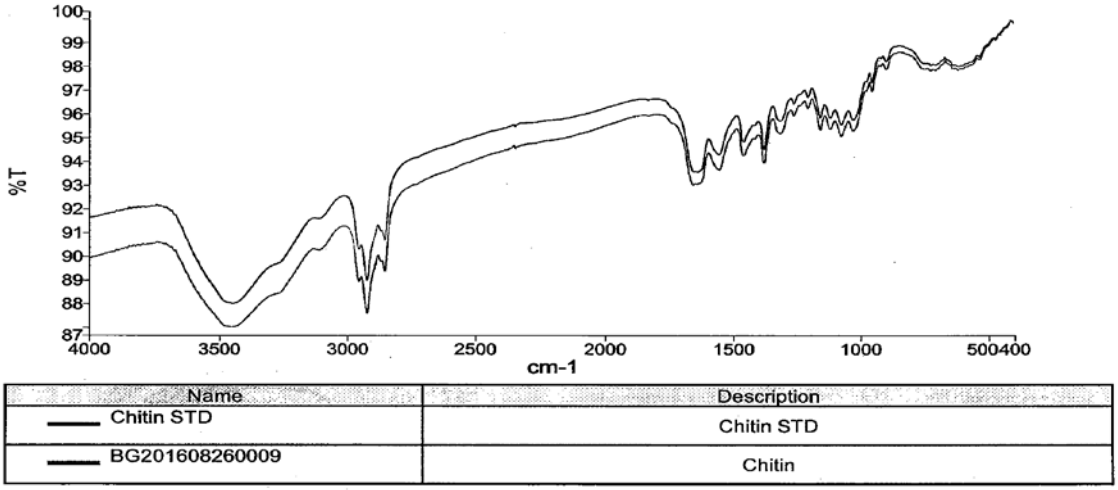


Figure 1: FTIR spectrum of chitin from button mushroom and standard chitin sample

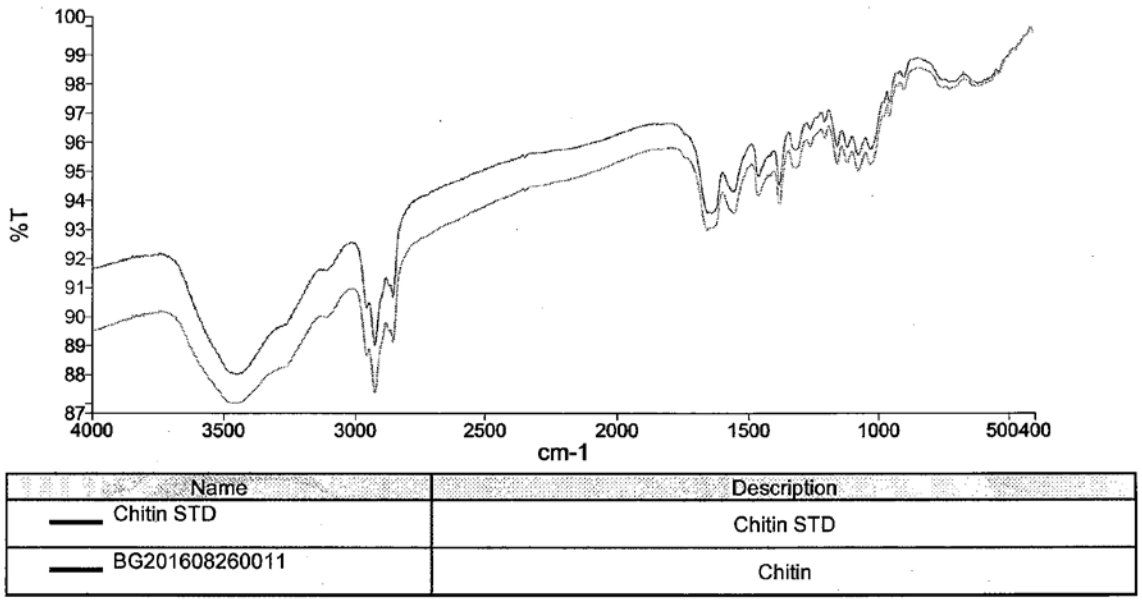
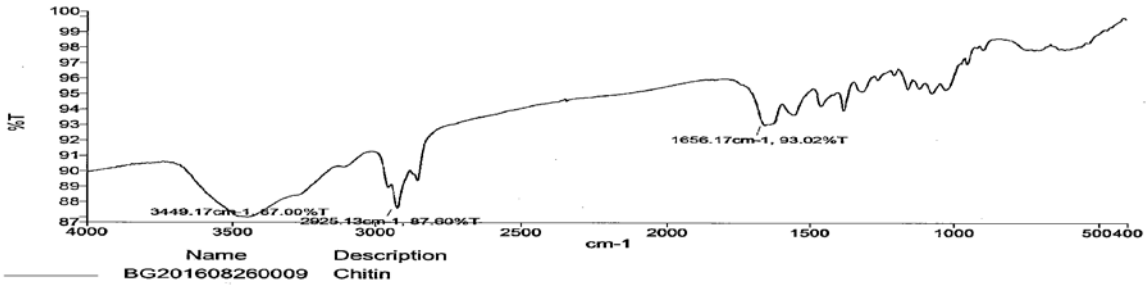


Figure 2: FTIR spectrum of chitin from oyster mushroom and standard chitin sample



Peak Table

Peak No.	X (cm-1)	Y (%T)	Peak No.	X (cm-1)	Y (%T)	Peak No.	X (cm-1)	Y (%T)
1	3451.36	88	2	2925.06	88.99	3	1639.1	93.57

Peak No.	X (cm-1)	Y (%T)	Peak No.	X (cm-1)	Y (%T)	Peak No.	X (cm-1)	Y (%T)
1	3449.17	87	2	2925.13	87.6	3	1656.17	93.02

Figure 3: FTIR spectrum of Standard chitin sample

Acid hydrolysis of oyster mushroom has yielded 1.5 % of chitin nanowhiskers from the net weight of fresh mushrooms while button mushroom yielded 2.2 %. At the end of sonication treatment, the sample had a consistency of a colloid. SEM images of the partially dried colloidal sample containing isolated chitin nanowhiskers from the mushrooms are illustrated in Figure 4 to Figure 7. The sample showed bundles of chitin nanowhiskers with length ranging from 50-200nm and width of ~11nm (Table 1). The size range of nanowhiskers isolated is similar to that of fibers isolated from crab shells [21].

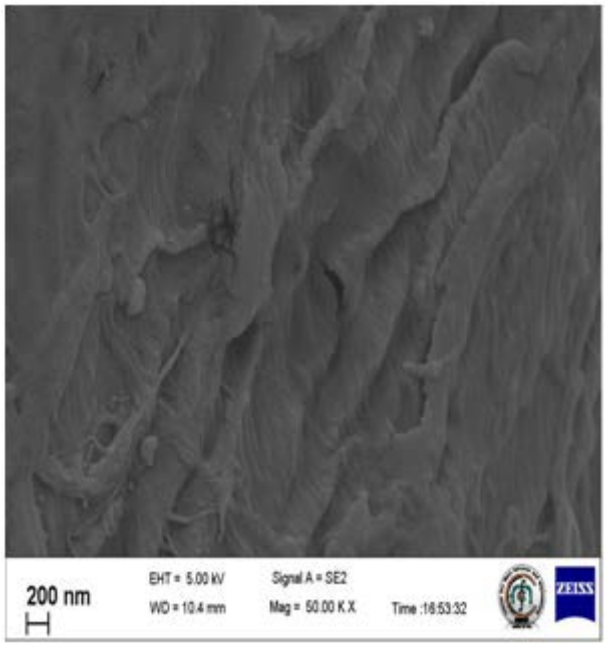


Figure 4: SEM images of chitin nanowhiskers from oyster mushroom at 200nm

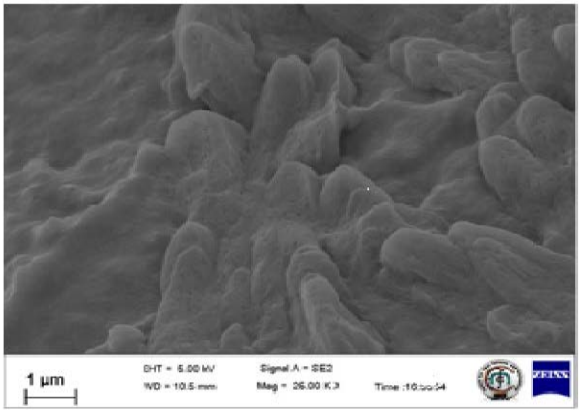


Figure 5: SEM images of chitin nanowhiskers from oyster mushroom at 1µm

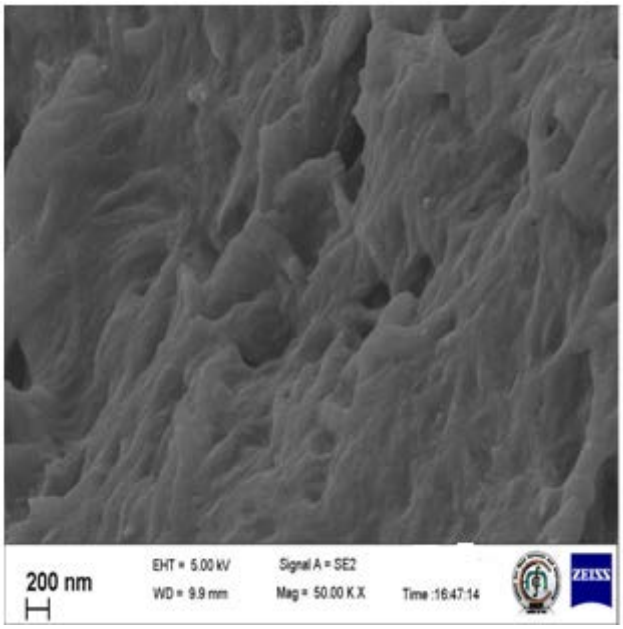


Figure 6: SEM images of chitin nanowhiskers from button mushroom at200nm

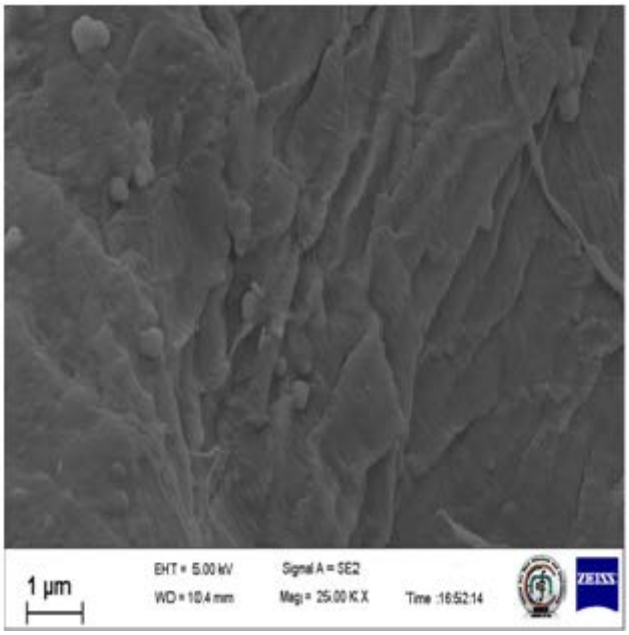


Figure 7: SEM images of chitin nanowhiskers from button mushroom at1µm.

Table 1: Comparative estimation of nanochitin in *Button* and *Oyster mushrooms*

Different Conc. of HCl	Absorbance at 555nm		Quantity present(mg/2g)		Percentage yield of chitin	
	Button mushroom	Oyster mushroom	Button mushroom	Oyster mushroom	Button mushroom	Oyster mushroom
3N	0.023	0.602	23	602	1.15	30.1
5N	0.005	0.656	5	656	0.25	32.8
7N	0.069	0.834	69	834	3.45	41.7
9N	0.286	0.672	286	672	14.3	33.6
12N	0.048	0.889	48	889	2.4	44.45
Average % yield					4.31	36.53

Table 2: Source and size of chitin nanowhiskers from mushrooms

Sl. No	Mushrooms	Extraction method	Length(nm)	Width(nm)
1	*Oyster mushroom*	Hydrochloric acid hydrolysis	50-200 to few microns	9-10.5 and ~11
2	*Button mushroom*	Hydrochloric acid hydrolysis	50-200 to few microns	8-11

5. DISCUSSION

Chitin is predominantly present as a fibrillar crystalline material. Based on infrared spectroscopy and X-ray diffraction data, native chitin is known to occur in one of the three crystalline forms α-chitin, β-chitin, and γ-chitin, respectively, depending on its origin [22]. The molecules in α-chitin are arranged in an antiparallel fashion, with strong intermolecular hydrogen bonding. The α- chitin is the most abundant form existing in the shells of crabs, lobsters, krill and shrimps, insect cuticle, and the cell walls of fungal and yeast [3] having a crystallinity higher than 80% [23]. In β - chitin, present in squid pens and tube worms [24], [25] where the chains are arranged in a parallel fashion. The γ -chitin is the form of chitin in which the molecules are arranged in both parallel and anti-parallel manner. The α –chitin is more stable when compared to the β -chitin and γ-chitin and hence more readily available in the market [26]-[27]. The chitin whiskers have a very strong potential for the development of nanomaterials because of their nano-sized structure, very high surface-to-volume ratio, high dispersibility in water and high viscosity, and excellent physical properties (otherwise called whisker i.e. highly crystalline chitin nanofibril) is composed of about 20 linear chains of N-acetylglucosamine, based upon the rod diameter and crystalline network dimensions[28]. Chitin content of oyster mushroom is 32.22% higher than that in button mushroom. Quality and characteristics of the chitin extracted from both mushrooms are at par with standard commercial chitin.

CONCLUSION

The current study has standardized the acid hydrolysis method for the isolation of chitin nanowhiskers from fresh edible mushrooms. Use of 9N and 12N HCl has been identified as ideal for extraction of nanowhiskers from the button and oyster mushrooms respectively. The average chitin content of oyster mushroom (*P. ostreatus*) is 32.22% higher than that of the button mushroom (*A. bisporus*).

REFERENCES

1. Li J, Revol JF, Marchessault RH, "Effect of degree of Deacetylation of Chitin on the Properties of Chitin Crystallites," J Appl Polym Sci., 65(2), pp. 373-380, 1997.
2. M. N. V. Ravi kumar, "A Review of Chitin and Chitosan Applications," Reactive and Functional Polymers, 46(1), , pp. 1-27,2000.
3. Marguerite Rinaudo, "Chitin and chitosan: Properties and applications" Prog. Polym. Sci., 31,pp 603–632, 2006.
4. Visakh PM, Thomas S, "Preparation of bionanomaterials and their polymer nanocomposites from waste and biomass," Waste Biomass Valorization, 1, pp 121–134, 2010.
5. Muzzareelli RAA. "Chitosan- based dietary foods," *Carbohydr. Polym*.,29 pp 309- 316, 1996.
6. Kadokawa J, Takegawa A,Mine S, Prasad K, "Preparation of chitin nanowhisker using an ionic liquid and their composite materials with poly(vinyl alcohol),"*Carbohydrate Polymers*., 84, pp1408, 2011.
7. Mera A, Araki J, Ohtsuki T, Shimosaka M, Yoshida N, "Chitin Nanowhiskers Mediate Transformation of Escherichia coli by Exogenous Plasmid DNA,"*J Biotechnol Biomaterial* ., 1, pp 114, 2011.
8. Wang JP, Chen YZ, Yuan SJ, Sheng GP, Yu HQ,"Synthesis and characterization of a novel cationic chitosan-based flocculant with a high water-solubility for pulp mill wastewater treatment," *Water Res*., 43(20), pp 5267-75, 2009.
9. Anchisi C, Meloni MC, Maccioni AM, "Chitosan beads loaded with essential oils incosmetic formulations," *J. Cosmet. Sci*., 57 pp 205-214, 2006.
10. Gautier S, Xhauflaire-Uhoda E, Gonry P, Piérard GE. "Chitin–glucan, a natural cell scaffold for skin moisturization and rejuvenation International" *Journal of Cosmetic Science*; 30(6): 459–469,*2008*.
11. Potara, M., Jakab, E., Damert, A., Popescu, O., Canpean, V., & Astilean, S, "Synergistic antibacterial activity of chitosan-silver nanocomposites on Staphylococcus aureus" Nanotechnology, 22, 2011.
12. Jiang X, Cai K, Zhang J, Shen Y, Wang S, Tian X, "Synthesis of a novel water-soluble chitosan derivative for flocculated decolorization," *J. Hazard Mater*., 185, 2-3,2011.
13. Jayakumar R, Prabaharan M, Sudheesh Kumar PT, Nair SV, Furuike T, Tamura H. Novel, "Chitin and Chitosan Materials in Wound Dressing, *Biomedical Engineering Trends in Materials Science,* ISBN: 978-953-307-513-6. 2011
14. Revol JF Marchessault RH. "Invitro chiral nematic ordering of chitin crystallites," Int. *J. Biol. Macromol.*, 15, pp 329-335. 1993.
15. Muzzarelli RAA. Natural chelating polymers. Oxford, New York, USA *Pergamon Press*., 83, 1973.
16. Muzzarelli, RAA. Chitin. Oxford, New York, USA *Pergamon Press, Oxford*., 309,1977.
17. Muzzarelli RAA, PierfrancescoMorganti , GianlucaMorganti ,Paolo Palombo , Marco Palombo , GraziellaBiagini , Monica Mattioli Belmonte ,Federica Giantomassi , FiorenzaOrlandi , CorradoMuzzarelli, "Chitin nanofibrils/chitosan glycolate composites as wound medicaments *Carbohydrate Polymers*" 70, pp 274–284, 2007.
18. Zikakis JP, "Chitin, Chitosan and Related Enzymes," Orlando, FL, USA *Academic Press*.,XVII. 1984
19. Singh DK, Ray, ARJ, Macromol. Sci.-Rev. *Macromol. Chem. Phys*., C40, 69, 2000

20. Ifuku S, Yamada K, Morimoto M, Saimoto H. Nanofibrilallation of dry chitin powder by star Burst System. *Journal of Nanomaterials*, 1155. 2012
21. Raabe D, Romano P, Sachs C, Al-Sawalmih A, Brokmeier HG, Yi SB, "Discovery of a honeycomb structure in the twisted plywood patterns of fibrous biological nanocomposite tissue," *Journal of Crystal Growth*., 283, pp 1–7, 2005.
22. Gardner KH, Blackwell J. "Refinement of the structure ofb-chitin," *Biopolymers*, 14, pp1581–95, 1975.
23. Fan Y, Saito T, Isogai A. "Chitin nanocrystals prepared by TEMPO mediated Oxidation of α-chitin. *Biomacromolecules*., 9, 192–198, 2008a.
24. Alain Dufresne. "Processing of Polymer Nanocomposites Reinforced with Polysaccharide Nanocrystals Molecules" 15, 4111-4128, 2010.
25. Peesan M, RujiravanitR, SupapholP, "Preparation and characterization of α-chitin whisker-reinforced chitosan nanocomposite," *Polymer Testing*., 22, 381, 2003.
26. Claramaría Rodríguez-Gonza, Ana L, Martínez-Herna, Víctor M, Castan, Oxana V, Kharissova, Rodney S,Ruoff, Carlos Velasco-Santos, "Polysaccharide Nanocomposites Reinforced with Graphene Oxide and Keratin-Grafted Graphene Oxide," *Ind. Eng. Chem. Res*.,51, pp 3619−3629, 2012.
27. Mincea M, Negrulescu A, Ostafe V, "Preparation, modification, and applications of chitin nanowhiskers," A Review, *Rev.adv.mater.sci*., 30, pp 225-242, 2012.
28. Ifuku S, Nogi M, Abe K, Yoshioka M, Morimoto M, Saimoto H, Yano H, "Preparation of chitin nanofibers with a uniform width as α-chitin from crab shells" *Biomacromolecules*., 10, pp 1584-1588, 2009.

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