

鳥取大学研究成果リポジトリ
Tottori University research result repository

タイトル Title	Preparation of chitin nanofibers by surface esterification of chitin with maleic anhydride and mechanical treatment
著者 Author(s)	Aklog, Yihun Fantahun; Nagae, Tomone; Izawa, Hironori; Morimoto, Minoru; Saimoto, Hiroyuki; Ifuku, Shinsuke
掲載誌・巻号・ページ Citation	Carbohydrate Polymers , 153 : 55 - 59
刊行日 Issue Date	2016-11-20
資源タイプ Resource Type	学術雑誌論文 / Journal Article
版区分 Resource Version	著者版 / Author
権利 Rights	(C) 2016. This manuscript version is made available under the CC-BY-NC-ND 4.0 license https://creativecommons.org/licenses/by-nc-nd/4.0/
DOI	10.1016/j.carbpol.2016.07.060
URL	https://repository.lib.tottori-u.ac.jp/6882

1 **Preparation of Chitin Nanofibers by Surface Esterification of Chitin**
2 **with Maleic Anhydride and Mechanical Treatment**

3
4 Yihun Fantahun Aklog^a, Tomone Nagae^a, Hironori Izawa^a, Minoru Morimoto^b, Hiroyuki
5 Saimoto^a, Shinsuke Ifuku^{a,*}

6
7 ^a Department of Chemistry and Biotechnology, Tottori University, 4-101 Koyama-minami,
8 Tottori 680-8552, Japan

9 ^b Research Center for Bioscience and Technology, Tottori University, 4-101 Koyama-minami,
10 Tottori 680-8553, Japan

11
12 *Corresponding author: Department of Chemistry and Biotechnology, Tottori University, 4-
13 101 Koyama-cho Minami, Tottori 680-8550, Japan. Tel and Fax: +81-857-31-5592, e-mail:
14 sifuku@chem.tottori-u.ac.jp

15
16 **ABSTRACT**

17 Esterification with maleic anhydride significantly improved the mechanical disintegration of
18 chitin into uniform 10-nm nanofibers. Nanofibers with 0.25 degrees of esterification were
19 homogeneously dispersed in basic water due to the carboxylate salt on the surface.
20 Esterification proceeded on the surface and did not affect the relative crystallinity. A cast film
21 of the esterified chitin nanofibers was highly transparent, since the film was free from light
22 scattering.

23
24
25 **Keywords:** Chitin; Nanofiber; Esterification with maleic anhydride

26
27
28 **1. Introduction**

29 A nanofiber is defined as a fiber with a diameter of less than 100 nm. Due to their
30 unique morphology, nanofibers are quite different from micron-sized fibers in their
31 dimensional, optical, and mechanical properties. Chitin is the second most abundant
32 biopolymer on earth that has *N*-acetyl glucosamine repeating units, and is the main
33 component of exoskeletons of crustaceans such as crab and prawn. An exoskeleton
34 has a hierarchical structure consisting of fine chitin nanofibers (Raabe et al., 2006;
35 Chen et al., 2008). Recently, chitin nanofibers were obtained by the mechanical

36 disintegration of commercial chitin powder extracted from the shell of red snow crab
37 (Ifuku et al., 2010). The nanofibers were highly uniform, with widths of
38 approximately 10 nm. Since commercial chitin powder consists of aggregates of
39 nanofiber, chitin was transformed into nanofibers by mechanical processing (e.g.,
40 high-pressure fluidizing (Kose et al., 2011; Ifuku et al., 2012; Ajoy et al., 2013), disk
41 milling (Ifuku et al. 2009; Ifuku et al., 2011), and ultrasonic breaking (Fan et al.,
42 2008b; Fan et al., 2012; Lu et al., 2013), powerful blender (Mushi et al., 2014; Mushi
43 et al., 2016). However, strong mechanical stress was required to disintegrate chitin
44 into nanofibers due to the strong hydrogen bonding generated between nanofibers. A
45 lot of energy was consumed to obtain nanofibers, raising their production cost. Thus,
46 the development of an efficient disintegration process is indispensable to the
47 commercial application of chitin nanofibers.

48 Chemical treatment can improve disintegration efficiency. For instance, Isogai et al.
49 reported chitin nanocrystals prepared by TEMPO-mediated oxidation of α -chitin (Fan
50 et al., 2008a). Carboxylate groups were formed by the oxidation of chitin at the C-6
51 primary hydroxyl group on the nanofiber surface, facilitating mechanical
52 disintegration with the assistance of the osmotic pressure effect. Iwamoto et al.
53 prepared lignocellulose nanofibers by the esterification of wood flour with maleic
54 anhydride (Iwamoto & Endo, 2015). Carboxylate groups were generated by the
55 esterification of hydroxyl groups at the C2, 3, and 6 positions of cellulose. The
56 carboxylate group significantly reduced the energy required to produce lignocellulose
57 nanofibers. Inspired by these previous works, we here studied the facile mechanical
58 disintegration of commercially available chitin powder into nanofibers by chemical
59 treatment with maleic anhydride. Since chitin also has hydroxyl groups at the C3 and
60 C6 positions, it would be esterified also by maleic anhydride. And generating a
61 carboxylate group on chitin enables the facile conversion of chitin into nanofibers by
62 the subsequent mechanical treatment.

63

64 **2. Experimental**

65 **2.1. Materials**

66 Chitin powder from crab shell with a 6.4% degree of deacetylation (DDA) was
67 purchased from Koyo Chemical Industry (Hyogo, Japan) and used without further

68 purification. Maleic anhydride was purchased from Nacalai Tesque (Kyoto, Japan)
69 and used as received.

70 **2.2. Esterification of α -chitin with maleic anhydride**

71 Chitin was esterified as described previously, with partial modification (Iwamoto &
72 Endo, 2015). The scheme for preparing the esterified chitin nanofiber is shown in Fig.
73 1. Dry chitin powder (6 g) from crab shell reacted with maleic anhydride (30 g) at 120
74 °C for 3.5 hours with occasional stirring. The reactant was collected by filtration and
75 washed with acetone and then with pure water thoroughly until the filtrate became
76 neutral. To neutralize the introduced maleate groups, approximately 10-15 mL of 1.0
77 M NaOH aqueous solution was added to the esterified chitin water dispersion until the
78 pH of the solution reached 11. The excess NaOH was then removed by washing with
79 pure water until the pH of the suspension became 7.8. The final concentration of the
80 esterified chitin dispersion was 1.29 wt%. The sample was stored in the refrigerator.
81 The experimental yield was estimated by gravimetric analysis.

82 **2.3. Mechanical disintegration**

83 The esterified chitin was diluted with water at 0.5 wt%, and the dilution was treated
84 with a grinder (MKCA6-3; Masuko Sangyo Co., Ltd., Kawaguchi, Japan) twice. The
85 grinder treatment was performed at 1500 rpm with a clearance gauge of -1.5
86 (corresponding to a 0.15 mm shift) from the zero position, which was determined as
87 the point of slightest contact between the grinding stones. In principle, there is no
88 direct contact between the stones due to the presence of chitin suspension.

89 **2.4. Film preparation**

90 Esterified chitin nanofiber dispersion was diluted with deionized water at 0.1 wt%
91 concentration. After removal of dissolved gases under vacuum, the aqueous
92 dispersion was casted on Teflon plates and dried in the oven at 50 °C until the dried
93 films detached from the plates by themselves.

94 **2.5. Characterization**

95 The zeta potential of aqueous esterified chitin nanofiber dispersion at 0.5 wt% was
96 measured using a laser-Doppler method apparatus (ELSZ-1000ZS, Otsuka Electronics,
97 Hirakata, Japan). At least five samples were measured to know the average value. For
98 field emission scanning electron microscopic (FE-SEM) observation, esterified chitin
99 nanofiber dispersion was diluted with an excessive amount of ethanol and dried in an
100 oven. The dried samples were coated with an approximately 2 nm layer of platinum

101 using an ion sputter coater and were observed by FE-SEM (JSM-6700F; JEOL,
102 Tokyo, Japan) operating at 2.0 kV. The light transmittances of 0.1 wt% nanofiber
103 dispersion and the self-standing films were measured using a UV-Vis
104 spectrophotometer (V550; JASCO, Tokyo, Japan). The degree of substitution (DS) of
105 the maleate group introduced onto the nanofiber surface was calculated from the C
106 and N weight percentages obtained from an elemental analyzer (Vario, EL III;
107 Elementar, Hanau, Germany) according to:

$$108 \quad C/12.01 : N/14.01 = (8 + 4n) : 1$$

109 where C and N are the weight percentages of carbon and nitrogen atoms obtained
110 from the elemental analysis and n is the molar ratio of the DS values of the introduced
111 maleate groups against the *N*-acetyl glucosamine unit of the nanofiber. Electrical
112 conductivity titration method was also applied to determine DS (Fan et al., 2008a).

113 Infrared spectra of the samples were recorded with an FT-IR spectrophotometer
114 (Spectrum 65, Perkin-Elmer Japan, Tokyo, Japan) equipped with an ATR attachment.
115 The X-ray diffraction profiles were obtained using X-ray goniometer scanning from
116 5° to 60° with Ni-filtered CuK α from an X-ray generator (Ultima IV; Rigaku, Tokyo,
117 Japan) operating at 40 kV and 40 mA. The crystallinity indices (CI) of the samples
118 were calculated from the ratio of the area of four crystalline peaks derived from (020),
119 (110), (120), and (130) plane to the total area from $2\theta = 5^\circ$ to 30° (Park et al., 2010).
120 The esterified chitin crystal sizes of the (020) and (110) planes were measured from
121 the widths at half heights of the diffraction peaks, using Scherrer's equation
122 (Alexander, 1979).

123

124 **3. Results and Discussion**

125 **3.1. Preparation of esterified chitin nanofiber**

126 Dry α -chitin powder was reacted with neat maleic anhydride at 120 °C for 3.5 hours
127 (Fig. 1). Although maleic anhydride is solid at room temperature, it melts above 52.6
128 °C, enabling a solventless reaction, which offers the advantages of low cost, ease of
129 purification, a high reaction rate, and environmental friendliness. It is known that acid
130 anhydride is preferentially introduced into high reactive amino group (Ifuku et al.,
131 2011). Thus, maleyl group was at first introduced into amino group, slightly existing
132 on chitin nanofiber in prior to hydroxyl group with lower reactivity. And then, maleic
133 anhydride could form ester linkages with primary hydroxyl group at C6 position

134 preferentially due to steric hindrance, thus forming a carboxylic acid group (-COOH).
135 After the reaction, the introduced carboxylic acid was neutralized by NaOH to change
136 it into carboxylate salt (-COO⁻Na⁺). The esterified chitin and unmodified chitin were
137 treated with the grinder twice to disintegrate them into nanofibers. After the
138 mechanical treatment, the esterified chitin was homogeneously dispersed in water,
139 and the dispersion was highly transparent compared to the unmodified chitin (Fig. 2).
140 The regular light transmittances of esterified and unmodified chitin dispersions in
141 water with a 0.5 wt% concentration at 600 nm were 91.5% and 13.1%, respectively.
142 To elucidate the dispersion property of esterified chitin nanofiber in water, the
143 dispersion was centrifuged at 10,000 rpm for 10 minutes. After the precipitate was
144 removed, the yield of nanofibers in the supernatant fraction was 97.2% in weight. The
145 high dispersion property comes from the surface property of the esterified chitin. The
146 average zeta potential of the esterified chitin dispersion at pH 7.8 was -48.41 ± 9.6
147 mV. The high negative surface charge is obviously attributable to the carboxylate
148 anion (-COO⁻). The strong negative charge enabled homogeneous dispersion. Original
149 chitin nanofiber with $+54.7 \pm 15.4$ mV zeta potential at pH 3.0 can disperse
150 homogeneously in acidic water. Slight amino group on the chitin enable stable
151 dispersion of nanofiber by electrostatic repulsion. On the other hand, the chitin
152 nanofiber precipitate in basic water immediately. Thus, esterified chitin with stable
153 dispersion in basic water allows for the chemical reaction under basic condition or
154 compounding with basic materials.

155 Figure 3a and 3b are SEM images of nanofibers obtained from esterified chitin. After
156 two cycles of simple disk milling treatment, the esterified chitin was easily and
157 completely disintegrated into homogeneous nanofibers over an extensive area. The
158 average thickness and length evaluated by image analyses of 50 randomly selected
159 nanofibers was 11.3 ± 4.4 nm and 275 ± 139 nm, respectively including a 2-nm-thick
160 platinum coating. The disintegration efficiency of the esterified chitin was much
161 higher than that of the unmodified chitin (Fig. 3c). Nanofibers in the unmodified
162 chitin were thicker and heterogeneous. The thicknesses varied widely from 10 to 100
163 nm. The average diameter was 45 ± 8 nm. The great difference in morphology
164 indicates that the carboxylate group of the esterified chitin helped the mechanical
165 disintegration. Anionic charges on the fiber surface brought about interfibrillar
166 electrostatic repulsion and osmotic pressure, facilitating mechanical disintegration.
167 Since nanofiber is defined as a fiber with a diameter of less than 100 nm and aspect

168 ratio of higher than 100, the esterified material may be more nanocrystal than
169 nanofiber.

170 **3.2. Characterization of esterified chitin nanofibers**

171 The degree of substitution (DS) of the maleate group of the esterified chitin
172 nanofibers, as determined by the elemental analysis of C and N atoms, was 0.25,
173 which means that one-fourth of the *N*-acetyl glucosamine unit was reacted with
174 maleic anhydride. The reaction was highly reproducible. For esterification, reaction
175 time and temperature strongly affected DS value. For example, when chitin was
176 esterified at 100 °C for 2 hours, the DS was 0.15. The difference in DS value affected
177 transparency of nanofiber dispersion. After the esterification, the weight of the chitin
178 increased to 6.17 g from 6.00 g. Since the theoretical weight of the esterified chitin
179 with 0.25 DS was 6.64 g, the percentage yield of the esterified chitin was 93% in
180 weight. This indicates that the reaction is suitable for producing chitin nanofibers
181 without significant mass loss during the process. Electrical conductivity titration
182 method was also applied to determine the DS. The DS was 0.21, which was slightly
183 lower than that from elemental analysis. The difference indicates that nanofibers were
184 completely esterified on the surface. Moreover, inner amorphous part of nanofiber
185 was also partially esterified.

186 FT-IR spectra of the esterified chitin nanofibers thus prepared are shown in Fig. 4.
187 The amide bands at 1652 cm⁻¹ and 1620 cm⁻¹, and the amide II band at 1556 cm⁻¹, are
188 characteristics of α -chitin. In addition, the peak at 1772 cm⁻¹ and the strong
189 overlapped peak at 1556 cm⁻¹ are derived from the C=O stretching vibration modes of
190 the maleate moiety. These newly appeared absorption bands are evidence of the
191 maleate introduced onto the nanofibers.

192 X-ray diffraction patterns of maleylated chitin nanofibers are shown in Fig. 5. The
193 diffraction peaks at approximately 9.4°, 19.5°, 20.7°, and 23.1° were in good
194 agreement with those of the typical antiparallel crystalline pattern of α -chitin, which
195 corresponds to (020), (110), (120), and (130) planes, respectively (Minke &
196 Blackwell, 1978). The relative crystallinity of esterified chitin nanofibers estimated
197 from the comparison between crystalline and total diffraction areas was 74%, which is
198 slightly higher than unmodified chitin (68%). The slight increase in the relative
199 crystallinity of chitin nanofiber may be attributed to the removal of amorphous part.
200 Maleic anhydride preferentially reacted with amorphous region and esterified

201 amorphous was removed by washing process. Moreover, maleate groups were
202 introduced onto the surface and the amorphous part of the nanofibers, and that the
203 original chitin crystalline structure was maintained after the esterification reaction.
204 The crystal sizes of the esterified nanofibers estimated by XRD patterns were 6.8 nm
205 and 5.4 nm at (020) and (110) planes, respectively.

206 We prepared a cast film using the maleated chitin nanofiber dispersion. The cast film
207 had a much higher transparency than that from the unmodified chitin nanofiber (Fig.
208 6). The regular light transmittances of the esterified and unmodified chitin nanofiber
209 films at 600 nm were 74.4% and 2.4%, respectively. The significant difference in
210 transparency is due to the differences in dispersibility in water and nanofiber
211 thickness between the two kinds of nanofibers. That is, esterified nanofibers that were
212 narrower and more dispersible in water were slowly concentrated by the evaporation
213 of the water so that nanofibers were piled up uniformly and gradually. The nanofibers
214 were so densely stacked that cavities in the cast film were almost completely removed.
215 Since the cast film was free from light scattering in the sheet, it became transparent
216 (Nogi et al., 2009).

217

218 **4. Conclusion**

219 Esterified chitin nanofibers with 0.25 DS were successfully prepared by the reaction
220 of chitin in neat maleic anhydride and subsequent mechanical disintegration without
221 losing its crystalline structure. The esterification significantly facilitated the
222 mechanical disintegration due to the strong electrostatic repulsion and osmotic
223 pressure. The surface carboxylate group enhanced the nanofibers' homogeneous
224 dispersion property in water at higher pH, which enabled us to obtain a highly
225 transparent film by a casting method. We expect that chitin nanofibers with an anionic
226 hydrophilic functional group, a uniform nanofiber morphology, and a very high
227 surface ratio will be available as a novel nano-biomaterial that can function in a
228 variety of applications.

229

230

231 **References**

232 Alexander, L. E. (1979). X-ray diffraction methods in polymer science. Ed. Krieger,
233 A. R., Huntington, New York.

234 Chen, P.-Y., Lin, A. Y.-M., McKittrick, J., & Meyers, M. A. (2008). Structure and
235 mechanical properties of crab exoskeletons. *Acta Biomaterialia*, *4*, 587–596.

236 Dutta, A. K., Yamada, K., Izawa, H., Morimoto, M., Saimoto, H., & Ifuku, S. (2013).
237 Preparation of chitin nanofibers from dry chitin powder by star burst system:
238 dependence on number of passes. *Journal of Chitin and Chitosan Science*, *1*, 59–64.

239 Fan, Y., Saito, T., & Isogai, A. (2008a). Chitin nanocrystals prepared by TEMPO-
240 mediated oxidation of α -chitin. *Biomacromolecules*, *9*, 192–198.

241 Fan, Y., Saito, T., & Isogai, A. (2008b). Preparation of chitin nanofibers from squid
242 pen β -chitin by simple mechanical treatment under acid conditions.
243 *Biomacromolecules*, *9*, 1919–1923.

244 Fan, Y., Fukuzumi, H. Saito, T., & Isogai, A. (2012). Comparative characterization of
245 aqueous dispersions and cast films of different chitin nanowhiskers/nanofibers,
246 *International Journal of Biological Macromolecules*, *50*, 69–76.

247 Ifuku, S., Nogi, M., Abe, K., Yoshioka, M., Morimoto, M., Saimoto, H., & Yano, H.
248 (2009). Preparation of chitin nanofibers with a uniform width as α -chitin from crab
249 shells. *Biomacromolecules*, *10*, 1584–1588.

250 Ifuku, S., Nogi, M., Yoshioka, M., Morimoto, M., Yano, H., & Saimoto, H. (2010).
251 Fibrillation of dried chitin into 10–20 nm nanofibers by a simple grinding method
252 under acidic conditions. *Carbohydrate Polymers*, *81*, 134–139.

253 Ifuku, S., Nogi, M., Abe, K., Yoshioka, M., Morimoto, M., Saimoto, H., & Yano, H.
254 (2011). Simple preparation method of chitin nanofibers with a uniform width of 10–
255 20nm from prawn shell under neutral conditions. *Carbohydrate Polymers*, *84*, 762–
256 764.

257 Ifuku, S., Miwa, T., Morimoto, M., & Saimoto, H. (2011). Preparation of highly
258 chemoselective *N*-phthaloyl chitosan in aqueous media. *Green Chemistry*, *13*, 1499–
259 1502.

260 Ifuku, S., Yamada, K., Morimoto, M., & Saimoto, H. (2012). Nanofibrillation of dry
261 chitin powder by Star Burst system. *Journal of Nanomaterials*, *2012*, 1–7.

262 Iwamoto, S., & Endo, T. (2015). 3 nm Thick lignocellulose nanofibers obtained from
263 esterified wood with maleic anhydride. *ACS Macro Lett.*, *4*, 80–83.

264 Kose, R., & Kondo, T. (2011). Favorable 3D-network formation of chitin nanofibers
265 dispersed in water prepared using aqueous counter collision. *Sen-I Gakkaishi*, *67*, 91–
266 95.

267 Lu, Y., Sun, Q., She, X., Xia, Y., Liu, Y., Li, J., & Yang, D. (2013). Fabrication and
268 characterisation of α -chitin nanofibers and highly transparent chitin films by pulsed
269 ultrasonication. *Carbohydrate Polymers*, *98*, 1497-1504.

270 Minke, R. & Blackwell, J. (1978). The structure of *a*-chitin. *Journal of Molecular*
271 *Biology*, *120*, 167–181.

272 Mushi, N. E., Butchosa, N., Salajkova, M., Zhou, Q., & Berglund, L. A. (2014).
273 Nanostructured membranes based on native chitin nanofibers prepared by mild
274 process. *Carbohydrate Polymers*, *112*, 255-263.

275 Mushi, N. E., Kochumalayil, J., Cervin, N. T., Zhou, Q., & Berglund, L. A. (2016).
276 Nanostructurally Controlled Hydrogel Based on Small-Diameter Native Chitin
277 Nanofibers: Preparation, Structure, and Properties. *ChemSusChem*, *9*, 989–995.

278 Nogi, M., Iwamoto, S., Nakagaito, A. N., & Yano, H. (2009). Optically transparent
279 nanofiber paper. *Advanced Materials*, *21*, 1595-1598.

280 Park, S., Baker, J. O., Himmel, M. E., Parilla, P. A., & Johnson, D. K. (2010).
281 Cellulose crystallinity index: Measurement techniques and their impact on
282 interpreting cellulase performance. *Biotechnology for Biofuels*, *3*, 1–10.

283 Raabe, D., Romano, P., Sachs, C., Fabritius, H., Al-Sawalmih, A., Yi, S.-B., Servos,
284 G., & Hartwig, H. G. (2006). Microstructure and crystallographic texture of the
285 chitin–protein network in the biological composite material of the exoskeleton of the
286 lobster *Homarus americanus*. *Material Science and Engineering: A*, *421*, 143–153.

287

288 **Figure Captions**

289 Fig. 1. Preparation scheme for the surface-maleated chitin nanofiber

290 Fig. 2. Photograph and UV-Vis spectra of (a) untreated and (b) maleated chitin
291 nanofiber dispersions

292 Fig. 3. SEM images of (a and b) esterified and (c) unmodified chitin nanofiber. Scale
293 bar: (a) 1000 nm, (b and c) 200 nm.

294 Fig. 4. FT-IR spectra of (a) unmodified and (b) maleated chitin nanofibers

295 Fig. 5. X-ray diffraction profiles of (a) unmodified and (b) maleated chitin nanofibers

296 Fig. 6. Appearances, SEM images, and UV-Vis spectra of (a) maleated and (b)
297 unmodified chitin nanofiber film. Scale bar: 200 nm

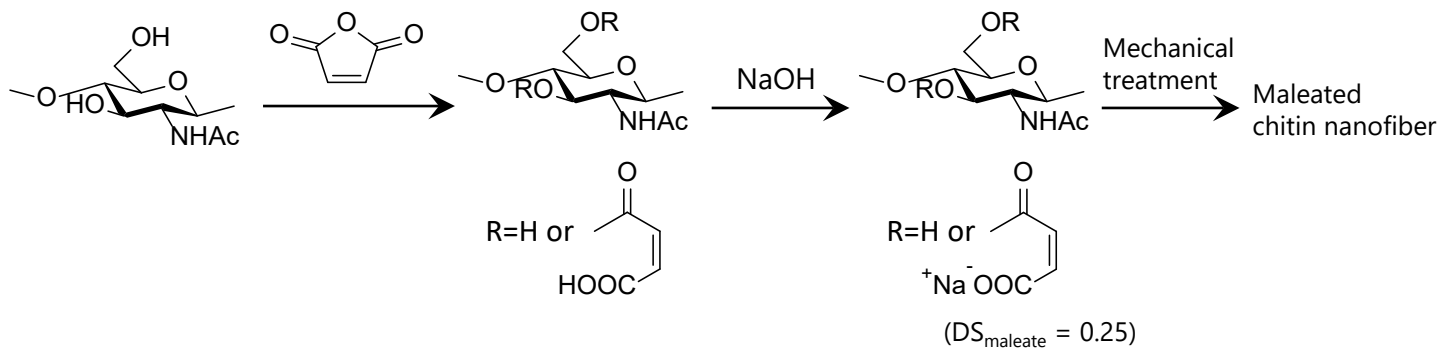


Fig. 1. Preparation scheme for the surface maleated chitin nanofiber

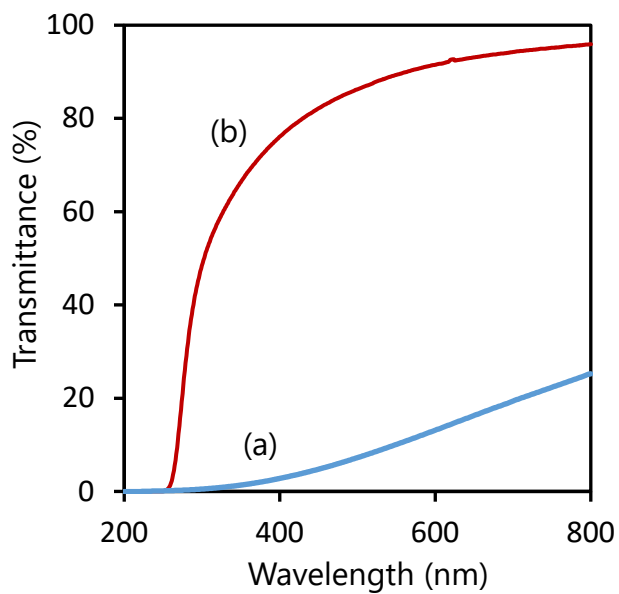
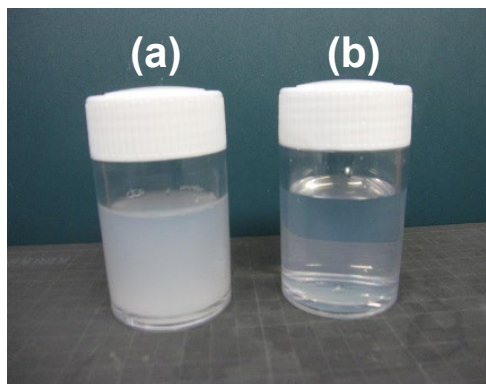


Fig. 2. Photograph and UV-Vis spectra of (a) untreated and (b) maleated chitin nanofiber dispersion

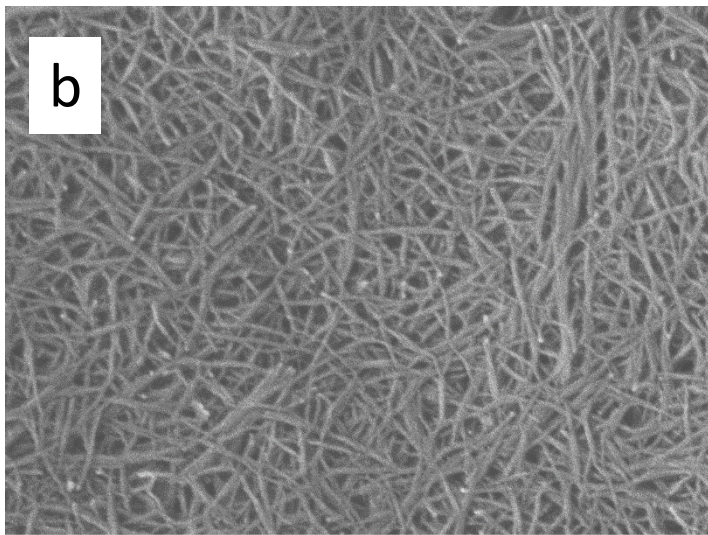
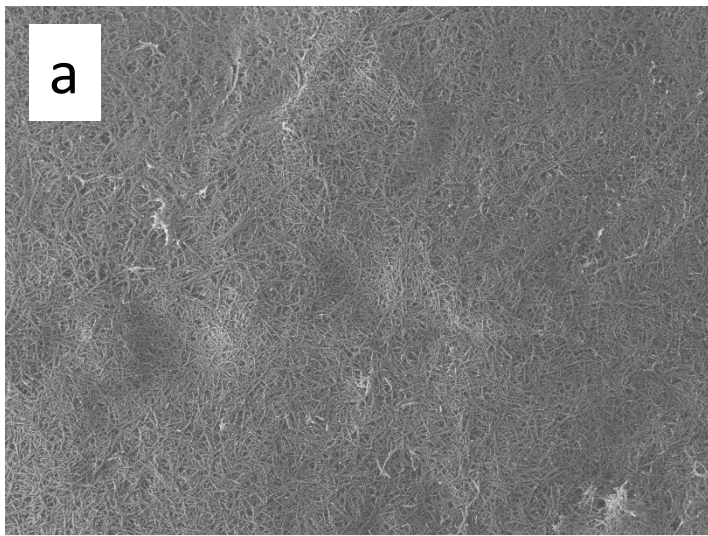


Fig. 3. SEM images of (a and b) esterified and (c) unmodified chitin nanofiber. Scale bar: (a) 1000 nm, (b and c) 200 nm.

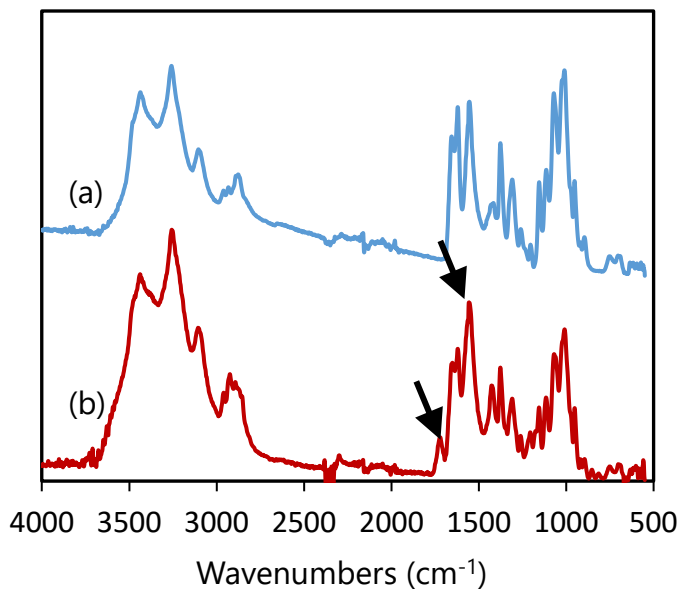


Fig. 4. FT-IR spectra of (a) unmodified and the (b) maleated chitin nanofiber

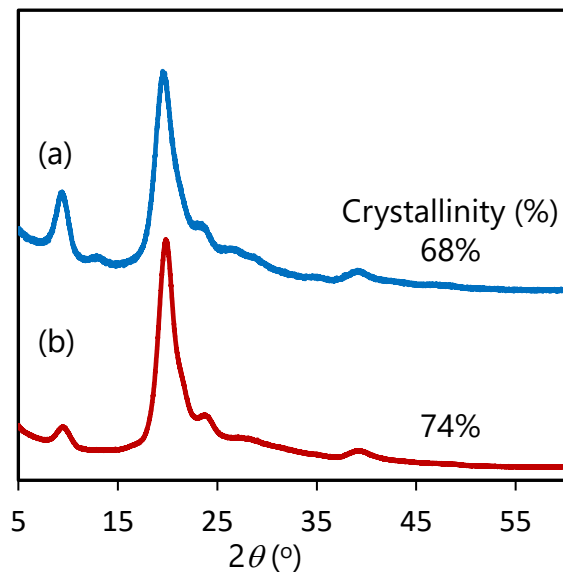


Fig. 5. X-ray diffraction profiles of (a) unmodified and the (b) maleated chitin nanofibers

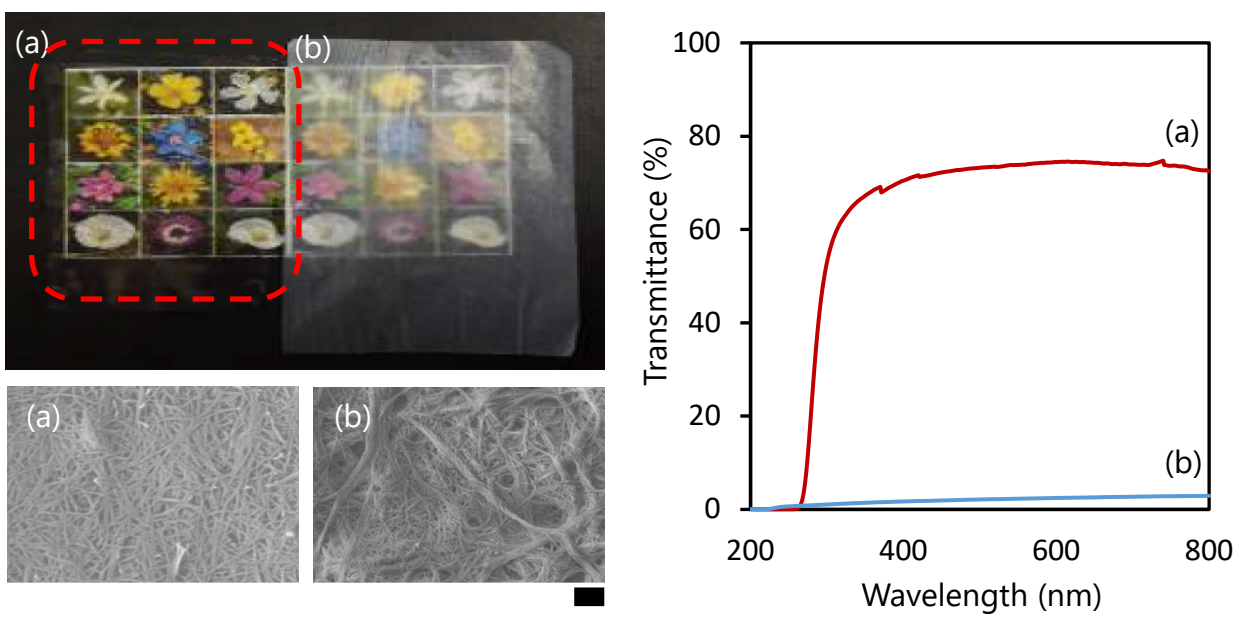


Fig. 6. Appearances, SEM images, and UV-Vis spectra of (a) maleated and (b) unmodified chitin nanofiber film