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Simple preparation method of chitin nanofibers with a uniform width of 10–20 nm from prawn shell under neutral conditions

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ABSTRACT

Chitin nanofibers were prepared from prawn shell by a simple grinding treatment after the removal of proteins and minerals. Since the exoskeleton of prawn is made up of a finer structure than crab shell, nano-fibrillation of prawn shell was easier than that of crab shell, which allow chitin nanofibers to be prepared under neutral pH conditions. The prepared chitin nanofibers were highly uniform and the width was 10–20 nm, which was similar to nanofibers from crab shell treated under acidic conditions. The preparation method for chitin nanofibers from prawn shells without an acidic chemical was applicable to many prawn species.

1. Introduction

Chitin is the second most widely occurring natural carbohydrate polymer next to cellulose and is synthesized in quantities of approximately 10^{10} to 10^{11} tons each year (Nair & Dufresne, 2003). It is the main component of the external skeleton of crabs, prawns, and insects. Recently, we succeeded in preparing α-chitin nanofibers from dried crab shells with a uniform width of approximately 10–20 nm (Ifuku et al., 2009). The crab shell has a strictly hierarchical organization consisting of crystalline α-chitin nanofibers and various types of proteins and minerals (Chen, Lin, Mckittrick, & Meyers, 2008). Because each chitin nanofiber is encased in embedding matrix components, the nanofibers can be isolated by removal of the matrix substances after a very simple mechanical treatment (Abe, Iwamoto, & Yano, 2007; Ifuku et al., 2009). Moreover, the mechanical treatment under acidic conditions is also the key to preparing chitin nanofibers. Cationization of amino groups in the chitin by the addition of an acid is important to maintain a stable dispersion state by electrostatic repulsions, which facilitate nano-fibrillation into chitin nanofibers (Fan, Saito, & Isogai, 2008). Because this method allows homogeneous chitin nanofibers to be obtained, we expect these nanofibers to be of rather uniform width with a high surface-to-volume ratio which can be developed into a novel green nanomaterial. However, in some cases, the acidic condition may cause significant problems for application of chitin nanofibers such as in biomedical materials, nanocomposites, electronics devices, and so on, because in general, these materials are sensitive to acid. However, it is difficult to remove an acidic chemical from chitin nanofiber suspension, since chitin nanofibers are homogeneously dispersed in water to give a high viscosity. Therefore, preparation of chitin nanofibers without an acidic chemical is required to expand their application. Here, we report on the extraction of α-chitin nanofibers from prawn shells under neutral conditions without the use of any acid.

2. Experimental

Fresh prawn shells of *Penaeus monodon* (black tiger prawn), *Marsupenaeus japonicus* (Japanese tiger prawn), and *Pandaluseous Makarov* (Alaskan pink shrimp) were purified to prepare the chitin nanofibers. Proteins and minerals were removed according to the conventional method using NaOH and HCl solutions, respectively (Shimahara & Takiguchi, 1988). About 80 g of wet prawn shells (dry weight was 20 g) was treated with 1 L of 2N HCl for 2 days at room temperature to remove the mineral salts. After filtration and rinsing with distilled water, the sample was refluxed in 1 L of 1N NaOH for 2 days to remove the proteins. The pigment component in the sample was then removed using 95% ethanol for 6 h under reflux followed by filtration and washing with water. The yield of dry chitin from the wet prawn shells was approximately 16.7% (black

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tiger prawn). Since drying process of chitin fibers generates strong hydrogen bonding between fibers, chitin must be kept wet after removal of the proteins and minerals.

The purified wet chitin was dispersed in water at 1 wt%, and the suspension was treated with a domestic blender. The slurry was passed through a grinder (MKCA6-3; Masuko Sangyo Co., Ltd.) at 1500 rpm. Grinding treatment was performed with a clearance gauge of −1.5 (corresponding to a 0.15 nm shift) from the zero position, which was determined as the point of slight contact between the grinding stones (Abe et al., 2007).

The suspension of chitin fibrils was dried at 105 °C after replacing the water with ethanol. The obtained sheets were coated with an approximately 2-nm layer of platinum by an ion sputter coater and observed with a field emission scanning electron microscope (JSM-6700F; JEOL, Ltd.).

The DS values of the amino groups of the chitin nanofibers were calculated from the C and N content in the elemental analysis data using an elemental analyzer (Elementar Vario EL III, Elementar).

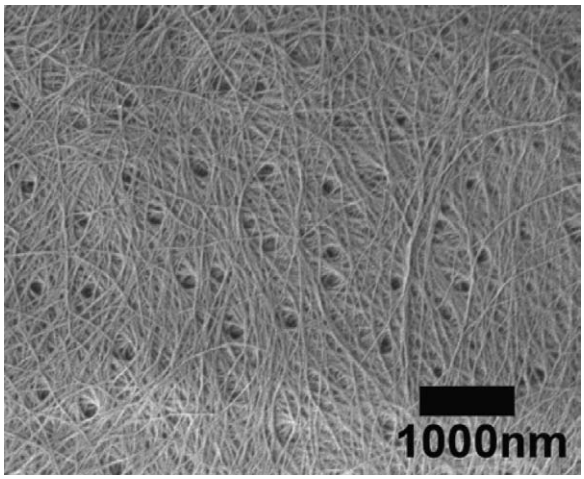

Fig. 1. FE-SEM micrographs of the surface of the black tiger prawn after removing matrix substances.

3. Results and discussion

Black tiger prawn shell was used as a starting material. It is widely cultured prawn species in the world and some of its shell is thrown away as industrial waste without effective utilization. Proteins and minerals were removed according to the conventional method using 2N HCl and 1N NaOH solutions, respectively to extract the chitin component from the prawn shell (Shimahara & Takiguchi, 1988). It is well-known that almost all of proteins and minerals can be removed by purification methods. The average degree of deacetylation of the samples determined by elemental analysis was 7%. Although the value was slightly higher than that from crab shell (5%), the difference might be caused by the purification process (Ifuku et al., 2009). Fig. 1 shows the SEM image of the prawn shell surface after removal of the protein and mineral matrix components (without grinding treatment). This image is from the exocuticle part of the prawn shell. Although the appearance of the prawn shell is still intact and has not yet been destroyed by the grinding equipment, we could observe extracted chitin nanofibers with a uniform width and elaborate design.

The purified chitin suspension with a concentration of 1 wt% was passed through a grinder for nano-fibrillation without any acid. The obtained chitin slurry was observed using SEM (Fig. 2). The chitin slurry thus obtained was viscous after a single grinding treatment, and the high surface-to-volume ratio of the nanofibers indicated that the chitin had been successfully fibrillated. In the case of crab shell, the widths of the fibers were widely distributed over a range from 10 to 100 nm (Fig. 2a, Ifuku et al., 2009). On the other hand, in the case of prawn shell, we could see uniform shape of the chitin nanofibers using the same extraction treatment. The chitin nanofibers were highly uniform over an extensive

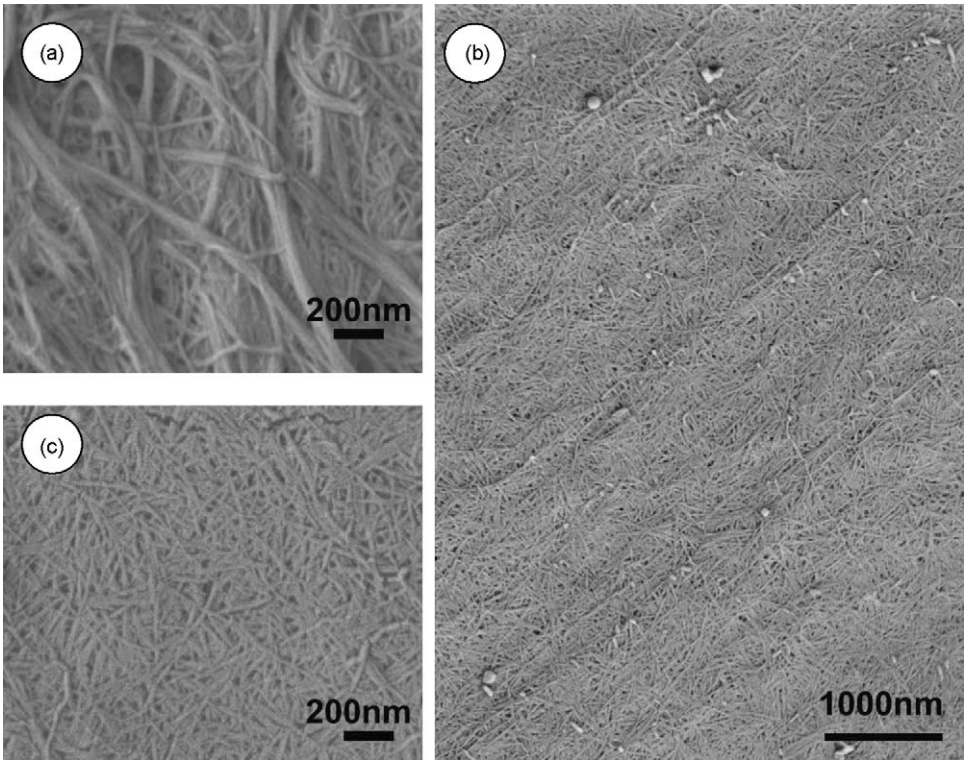

Fig. 2. FE-SEM micrographs of chitin nanofibers (a) from crab shell, (b and c) from black tiger prawn shell after one passage through the grinder under neutral pH conditions.

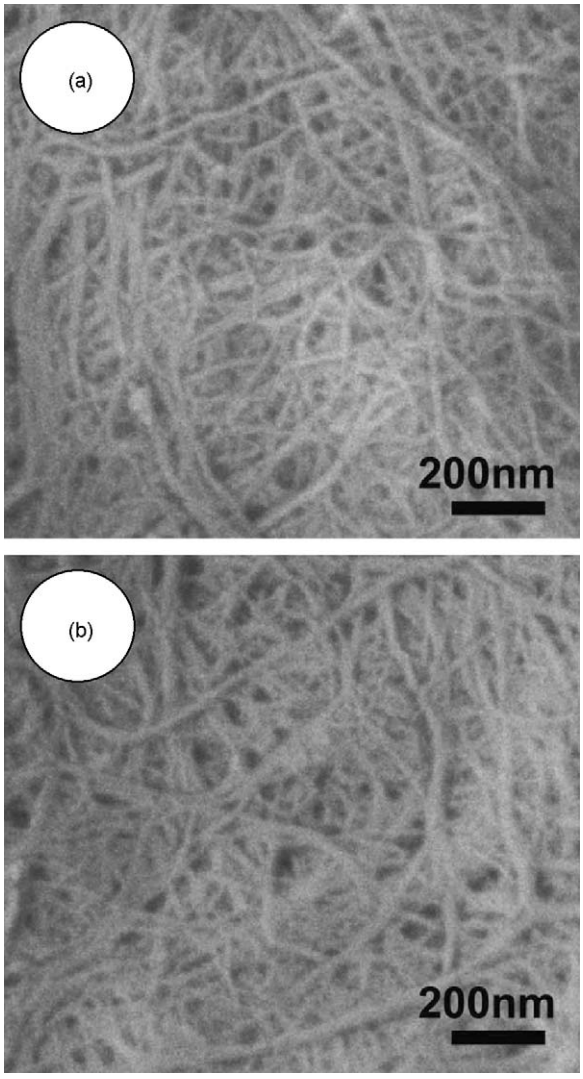

Fig. 3. FE-SEM micrographs of chitin nanofibers (a) from Japanese tiger prawn shell, and (b) from Alaskan pink shrimp shell after one pass through grinder under neutral pH condition.

area (Fig. 2b), and the width of the nanofibers was approximately 10–20 nm, including a 2 nm thick platinum coating layer (Fig. 2c). The fiber thickness of 10–20 nm was similar to nanofibers from crab shell fibrillated under an acidic condition, as described previously (Ifuku et al., 2009). Thus, chitin nanofibers were successfully prepared from prawn shell with a uniform width under a neutral pH condition.

The explanation of successful fibrillation could be as follows. The exoskeleton of crustaceans is made up of mainly two parts, the exocuticle and the endocuticle. Although the exocuticle has a very fine twisted plywood-type structure, the endocuticle has a much coarser matrix structure with a thicker fiber diameter. In general, approximately 90% of a crab shell is made up of the endocuticle (Chen et al., 2008). In contrast, the exoskeleton of Natantia, which has a semitransparent soft shell, including black tiger prawn, is made up primarily of a fine exocuticle, as shown in Fig. 1 (Yano, 1977). As a result, due to the differences in the cuticle structure and fiber thickness, fibrillation of prawn shell is easier than that of crab shell.

The preparation method for chitin nanofibers from prawn shells without an acidic chemical was found to be applicable to other prawns. Fig. 3 shows SEM images of the chitin nanofibers derived from Japanese tiger prawn and Alaskan pink shrimp, both of which are important food sources. These chitin nanofibers were prepared by the same procedure, that is, removal of matrix components and subsequent grinding treatment under a neutral pH condition. Both chitins are also observed as uniform nanofibers with a width of 10–20 nm, which is similar to the nanofibers from black tiger prawn. This result suggests that chitin nanofibers can be obtained from other prawn species having a very fine exocuticle structure by nano-fibrillation under neutral pH conditions.

4. Conclusion

Chitin nanofibers were prepared from a variety of prawn shells with a uniform width of approximately 10–20 nm by conventional chemical treatment, followed by mechanical treatment. In this study, application of prawn shell, which has a fine hierarchical structure, is the key to isolating homogeneous chitin nanofibers without any acid. Since many materials are sensitive to acid under a work process, this study will expand the application of chitin nanofibers as an environmentally friendly bionanomaterial with a very high surface-to-volume ratio.

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