Characterization of Nanofibers from Japanese Orange Inner Peels Prepared Using Pectinase and Diluted Alkali

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To develop the extracted nanofibers from wasted citrus peels, the preparation of cellulose nanofibers (CNFs) from Japanese citrus peels was investigated by using pectinase and the obtained nanofibers were characterized in the morphology and other properties in this study. First, the combination of pectinase treatment and diluted alkali treatment were applied as the pretreatment for mechanical nanofibrillation of Japanese citrus peels. Second, the obtained nanofibrillated peels were characterized and compared in the surface morphology. It was relatively easy to fibrillate cellulose from the Japanese citrus inner peels subjected to pectinase compared with cellulose obtained from other materials such as woody pulp. Nanofibers derived from citrus inner peels were easier to be mixed with the oil and maintained the small-size of oil drops more easily than the CNF derived from hardwood pulp. Considering these characteristics, the nanofibrillated cellulose obtained from citrus peels using pectinase could be applied as an emulsion stabilizer in the food and cosmetic industry.

Key Words
Japanese orange inner peels, Cellulose nanofibers, Emulsion stabilizer

1. Introduction

Cellulose nanofibers (CNFs) have attracted much attention as a promising advanced material 1). The preparation techniques and applications have been researched and developed using wood pulp. For example, nanofibers are not only used for support in constructing materials and for their gas-barrier function in packed materials but also in food and cosmetic materials for their moisture maintaining function and viscosity 2).

The annual worldwide orange production is 2.2 × 10^6 metric tons that resulted in an annual wastes of approximately 1.1 × 10^6 metric tons 3). Processing of large amount of wastes is costly and has led to environmental concerns 4). In Japan, orange juice processing mills are found in citrus cultivated areas such as in the Ehime, Wakayama, and Oita prefecture. Therefore, processing of significant amounts of wastes generated has created problems similar to those mentioned above. Especially, a very famous factory of Japanese orange juice is located in Ehime Prefecture. The most abundant species in Japanese orange peel wastes is Citrus unshiu, followed by C. iyo. These peels contain oils, pectin, cellulose, and hemicellulose 5). Oils and pectin are useful and have various applications. While, cellulose and hemicellulose in peels are required for effective applications. In the previous study, the CNFs from the inner peels of C.
were obtained by combining pectinase treatment and mechanical fibrillation using a grinder. The inner peels of *C. unshiu* were more easily nanofibrillated than wood pulp. However, the processability of other Japanese orange peels remains unknown. Moreover, some kind of cleaning process is required because the obtained CNFs from *C. unshiu* contained contaminants that may have been the degradation products of pectin and hemicellulose.

To extract clean CNFs from peels of *C. unshiu* and *C. iyo* Japanese oranges, CNFs from the inner peels of Japanese oranges were prepared using pectinase and dilute alkali. The morphology and solid-state properties of the obtained CNFs were characterized in this study.

2. Experimental

2.1 Materials

Inner peels of Japanese oranges (*Citrus Iyo* and *C. unshiu*) were kindly supplied by Ehime Inryo Co., Japan (Fig. 1). The both inner peels were washed with tap water, and wiped to remove the water.

2.2 Preparation of nano-fibrillated celluloses

Nanofibrillated Japanese citrus peels were prepared by modified the method previously reported. Wet inner peels of *C. iyo* or *C. unshiu* (theoretical dry weight of nearly 80 g), pectinase (Pectinase PL Amano, Amano Enzyme Co., Nagoya, Japan) diluted with 100 mM acetate buffer, and distilled water were all mixed in a 1 L flask. The flask containing the mixtures was incubated at 48 °C and 150 rpm for 22-24 h. After pectinase treatment, sodium hydrate was added into the flask, adjusted to 1% NaOH solution and autoclaved at 121 °C for 60 min. The reaction mixtures were neutralized, washed with distilled water and filtrated. The two obtained residues (*C. iyo* and *C. unshiu*) were fibrillated in the distilled water by using a high-speed blender (Absolute mill, Osaka Chemicals Co., Osaka, Japan) for obtaining approximately 1 wt% slurry, respectively. The slurry of *C. iyo* was fibrillated further if required using a grinder (MKZA6-2, Masuko Sangyo Co., Ltd., Saitama, Japan) for one time. A schematic flowchart of the process for preparing the nanofibrillated fibers from Japanese citrus peels is shown in Fig. 2. The obtained nanofibrillated peels were characterized and their surface morphology, thermal degradation properties, and their crystallinities were compared.

For comparison, the hardwood pulp was also nanofibrillated as described above. Dried hardwood pulp (10 g) was soaked in 800 ml pure water and blended for 3 min by a high-speed blender. Pure water (200 ml) was added to the blended samples and the mixture was ground four times using a grinder.

2.3 Field-emission scanning electron microscopy (FE-SEM)

The samples were prepared for FE-SEM observation based on the method previously reported. The samples were solvent-exchanged from water to ethanol and t-butanol, and freeze dried. The dried sample was coated with Pt using auto fine coaters (JFC-1600, JEOL Co., Japan). The observation was conducted by using FE-SEM machine (JSM 6700 FE-SEM, JEOL Co., Akishima, Japan) at 1.4-5.0 kV.

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**Fig. 1 Photographs of** (a) fruit, (b) cut plane of fruit, and (c) inner peels of *C. iyo*, and (d) fruit, (e) an uncovered piece, (f) an inner peel of *C. unshiu*
2.4 Stability of oil in the nanofibrillated fiber slurry

Squalane oil was added into an approximately 1% slurry of the nanofibrillated inner peels of *C. iyo* or the nanofibrillated hardwood pulp and adjusted to a final concentration of 1 wt%. The mixture was strongly agitated by using an absolute mill for 3 min to prepare emulsions, and left at rest for more than 3 days. The prepared emulsion-like mixtures were observed by using a digital microscope (KEYENCE, Co., Osaka, Japan).

3. Results and Discussion

FE-SEM was used to observe the surface of inner peels of *C. iyo* without pectinase: the fibers were barely detected (Fig. 3 (a)). This morphologic character is very similar to the surface of Japanese mikan (*C. unshiu*) inner peel described in a previous report. The apparent shape was sponge-like, however, many nanofibers covering amorphous blobs were recognized after careful observation. After pectinase treatment for 24 h, many nanoscale fibers called cellulose microfibrils, were clearly observed (Fig. 3 (b) and (c)) although fibers in width from millimeters to micrometers were reduced. Most nanofibers were between 20-50 nm wide, however, some nanofibers had aggregated and were more than 100 nm (Fig. 3 (c)).

These bundles of fibers before fibrillation was slightly thicker than the bundle derived from the inner peels of *C. unshiu*. However, fibers were thinner than fibers from woody pulp. Blending was attempted based on the method in the previous report, however, some aggregates of nanofibers remained, and some sheets made from these aggregates were observed (Fig. 3 (d)). In the previous report, these nanofibers sheets were rare on the surface of the inner peel after pectinase treatment. Probably, the aggregation of nanofibers on citrus inner peels depends on the species. Moreover, the properties of other inner peel (*C. iyo*) differ each cultivation year (data not shown). These nanofibers aggregated sheets were fibrillated after grinding, although a few bands of nanofibers aggregates remained (Fig. 3 (d)). Similar trends were observed for inner peels of *C. natsudaidai*, another kind of Japanese orange, but cellulose from this kind was slightly easier to fibrillate (data not shown). Throughout the whole process observing citrus inner peels, nanofibers could be observed after pectinase treatment and they was easier to fibrillate to nano-scale than woody fibers, however, their aggregation and the width of nanofibers aggregated bundles differed slightly depending on the species.

The relatively homogeneous and thin nanofibers were obtained by the combination of pectinase, diluted alkali, and blender treatments from inner peel of *C. unshiu* (Fig. 4 (a)). The relatively thin nanofibers were occasionally obtained from the inner peels of *C. iyo*, however, they were sometimes partially packed and formed into nanomesh sheets (Fig. 4 (b)). By grinding the inner peels of *C. iyo* fibrillated using a blender, the relatively thin nanofibers were obtained similarity of the *C. unshiu* although some bundles measured on the scale of few hundred nm (Fig. 4 (c)). Hardwood pulp ground four time is shown in Fig. 4 (d) as reference. The fibers width was of the submicron to nanoscale. These results suggest that the pectinase-treated Japanese citrus inner peels are easier to nanofibrillate than wood pulp, and the obtained nanofibers are relatively thin although the difficulty of the nanofibrillation is various and depended on the species and individual products.

The mixtures of the nanofibrillated inner peels of *C. iyo* or bleached hardwood pulp and squalane oil are

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**Fig. 3** FE-SEM images of raw inner peels of *C. iyo* without pectinase (a), and with pectinase (b and c), and frozen inner peels of *C. iyo* without pectinase (d), and with pectinase (e and f). Scale bars of (a) and (b) are 1 μm and those of (c)-(e) are 200 nm. White arrows of (d) and (f) represent some aggregates of nanofibers covered by pectic polysaccharides, and contaminants, respectively.
shown in Fig. 4. The oil droplets having a smaller size were more stabilized in the nanofibrillated inner peels of C. 
*iyo* suspension than in the nanofibrillated hardwood pulp. 
This could be because the width of the CNFs derived from 
Japanese citrus inner peel was relatively thin compared to 
that of CNFs from hardwood pulp (Fig. 5), and the CNFs 
from citrus inner peel had the small amounts of non-
cellulosic substances such as degraded pectin, hemicellulose 
and pigments (carotenoids).

It has been reported that the soybean oil droplets 
are stabilized in water by the network of microfibrillated 
cellulose from mangosteen 9). They have suggested that 
MFC provides the emulsion stability by forming a solid 
nanofibers network such as in a pickering emulsion, and 
has the potential application as an emulsion stabilizer 8). Our 
results are in agreement with these reports, and suggest 
that the nanofibrillated Japanese citrus inner peels prepared 
using pectinase also have the O/W emulsion stability and 
could be used as an emulsion stabilizer in the food and 
and cosmetic area.

The cellulose microfibrils obtained by grinding 
wise-oxidized residues have been reported to have almost 
the same in physical properties 9). While, it has also been 
reported that the cellulose microfibrils derived from fruit 
parenchymatous cells are originally thinner than those 
of wood 10). The nano-fibrillated celluloses prepared by 
using enzymes had different properties in this study. 
Probably, this was because a slight amount of non-cellulosic 
substances had remained. The utilization of enzymes for 
nanofibrillation has the advantages of an energy-saving, 
environmentally friendly and simple process 11) that is a 
highly effective pretreatment for fibrillation, however, it also 
has some disadvantages such as the difficulty in obtaining 
highly purified cellulose and some cellulosic damages such 
as molecular-scale ravels. Therefore, the applications of 
nano-fibrillated celluloses prepared using enzymes need to 
be proposed considering their characteristics. The nano-
fibrillated Japanese citrus peels using pectinase may be 
suitable for food and cosmetic applications.

4. Conclusion

The nano-fibrillated citrus inner peels prepared 
using pectinase were characterized in this study. It was 
ford that the nanofibrillation of Japanese citrus peels by a 
combination of pectinase and mechanical treatments was 
relatively easy compared with the nanofibrillation of woody 
pulp. These properties are important and would be key 
factors for the application of organic natural nanofibers.

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References
1) Yano, H., *Funct. Pap. Res. J.*, 49, 15-20 (2010)
2) Isogai, A.; Saito, T.; Fukuzumi, H., *Nanoscale*, 3, 71-85 (2011)
3) Rodriguez-Lope, A. D.; Mayor, L.; Galfarsoro, M.M.; Martinez-Otalo, J.; Garcia-Castello, E. M., *Agricultural Science*, 4, 45-50 (2013)
4) Hideno, A.; Abe, K.; Yano, H., *J. Food Science*, 79, N1218-N1224 (2014)
5) Maran, J.P.; Sivakumar, V; Thirugnanasambandham, K; Sridhar, R., *Carbohydrate Polymers*, 97, 703-709 (2013)
6) Abe, K.; Yano, H., *Carbohydrate polymers*, 85, 733-737 (2011)
7) Winuprasith, T.; Suphantharika, M., *Food Hydrocolloids*, 43, 690-699 (2015)
8) Winuprasith, T.; Suphantharika, M., *Food Hydrocolloids*, 32, 383-394 (2013)
9) Abe, K.; Yano, H., *Cellulose*, 16, 1017-1023 (2009)
10) Niimura, H.; Yokoyama, T.; Kimura, S.; Matsumoto, Y.; Kuga, S., *Cellulose*, 17, 13-18 (2010)
11) Pääkko, M.; Ankerfors, M.; Kosonen, H.; Ahola, A. N. S.; Osterberg, M.; Ruokolainen, J.; Laine, J.; Larsson, P.; Ikkala, O.; Lindstrom, T., *Biomacromolecules*, 8, 1934-1941 (2007)