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Chitin nanofiber as a promising candidate for improved salty taste

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ABSTRACT

In this study, we prepared chitin nanofibers (CNFs) of varying diameter and added sodium chloride (NaCl) to CNF solutions. We performed ultrasonication on the CNFs for 30, 45, and 60 min, following which, the CNFs (i.e., CNF30, CNF45, CNF60) displayed a diameter of 9.3, 5.6, and 5.1 nm, respectively; the diameter decreased with the ultrasonication time. The zeta potential of CNFs with various concentrations and diameters ranged from 21.8 to 37.3 mV. The kinematic viscosities of the CNFs were affected with the CNF concentrations and addition of NaCl. When 0.15 and 0.30 g/L CNF solutions were added 3.0 g/L NaCl, they tasted saltier than the 3.0 g/L NaCl solution, but less astringent compared with the CNF solutions without NaCl. The saltiness enhancement of CNF60 was superior to that of the CNF30 and CNF45 due to CNF60 was smaller diameter and less intertwined. The $-NH_3^+$ of CNF bound Cl^- by electrostatic interactions that causing the ratio of free Na^+ in the diffuse layer increased and then improving the saltiness and decreasing the astringency of the solutions. Therefore, application of CNFs in food is promising.

1. Introduction

Chitin is common in the exoskeletons of arthropods, squid pen, and cell walls of fungi and yeast. It is a long-chain polysaccharide with β-1,4 glycosidic bonds and comprises monomers of 2-acetamido-2-deoxy-β-D-glucose. Chitin is biodegradable, biocompatible, nontoxic and environmentally friendly biomaterials, which is applied in many fields such as agriculture, food and nutrition, biomedicine, biochemistry, cosmetics, textiles, materials science, and wastewater treatment (Hsieh, Chin, & Tsai, 2015).

Chitin nanofibers (CNFs) are formed by embedding chitin nanofibrils (diameter: approximately 2–5 nm, length: 300 nm) in protein matrices. Between CNF chains are also connected by strong hydrogen bonds (Ifuku, Yamada, Morimoto, & Saimoto, 2012). In general, CNFs are prepared by removing proteins and minerals from shrimp and crab shells and then applying certain physical or chemical methodologies to the shells. Common CNF-manufacturing methods include electrospinning (Barber, Griggs, Bonner, & Rogers, 2013), TEMPO-mediated oxidation (Fan, Saito, & Isogai, 2008), acid hydrolysis (Goodrich, & Winter, 2007), grinding (Ifuku et al., 2010), the star burst system (Ifuku et al., 2012), dynamic high-pressure homogenization (Salaberria, Fernandes, Diaz, & Labidi, 2014), microfluidization (Mushi, Butchosa, Salajkova, Zhou, & Berglund, 2014), and ultrasonication (Lu et al., 2013). Ultrasonication is one of the most effective methods for fabricating nanofibers, which involves utilizing acoustic cavitation to generate local hot spots that feature characteristics of high temperature (>5000 K), high pressure (>20 MPa), and a heating/cooling rate of $>10^{10}$ K/s. Such extreme environments disintegrate the strong interfibrillar hydrogen bonds in chitin molecular chains, enabling CNF extraction (Lu et al., 2013).

Chitins neither dissolve nor become dispersed in water. Chitin reduced to CNFs increase their dispersibility in water, forming a colloidal solution. At pH < 7, the surface of CNFs is positively charged (Fan, Fukuzumi, Saito, & Isogai, 2012; Fan et al., 2008; Pereira, Muniz, & Hsieh, 2014). When NaCl is added to a CNF solution, NaCl dissolves in the solution, forming Na^+ and Cl^-. Because of static electricity, $-NH_3^+$ on the CNF surface adsorbs Cl^-, forming a Stern layer; any remaining ions that are not adsorbed are randomly distributed in the solution, forming a diffuse layer. Principally, most Na^+ are distributed in the diffuse layer. When NaCl exceeds

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5 mmol/L, dispersion of 1.8% chitin nanocrystals is dominated by elastic characteristics, whereas salt-free dispersion is dominated by viscous characteristics (Tzoumaki, Moschakis, & Biliaderis, 2010).

Saltiness is primarily created by Na^+, and NaCl is the most commonly used salty agent. Na^+ and Cl^- are essential nutrients to the body and serve the functions of maintaining blood volume and blood pressure as well as regulating body fluid volume. The World Health Organization (2007) recommended a daily Na intake of less than 86 mmol (<5 g of NaCl); however, the average daily Na intake in the United States and Asia is 140–160 mmol (approximately 8.2–9.4 g of NaCl) and more than 206 mmol (≥12.0 g of NaCl), respectively (Liem, Miremadi, & Keast, 2011). Lawrence, Salles, Septier, Busch and Thomas-Danguin (2009) noted that excessive Na intake causes various problems such as increased blood pressure, gastric cancer, obesity, increased risk of cardiovascular disease, and reduced bone density. Therefore, many low-salt foods have been developed. Nevertheless, reducing the amount of salt influences the taste. Thus, a challenge facing the food industry is to reduce the amount of salt added to food without changing consumers' acceptance of low-salt foods (Lawrence et al., 2009).

Current salt-intake reduction methods include gradually reducing the salt level over time (Girgis et al., 2003), using substitutes such as KCl, $CaCl_2$, and $MgSO_4$, as well as adding flavor enhancers such as citric acid (Rolls, 1999), spices, and monosodium glutamate (Liem et al., 2011).

In the present study, CNFs with a low astringent taste were utilized to adsorb Cl^- and increase the ratio of free Na^+ in a solution to enhance the saltiness while reducing the amount of salt in foods. CNFs of varying diameters were prepared by adjusting the ultrasonication time. NaCl was then added to the CNF solution before the zeta potential, kinematic viscosity, and gustatory perception (sensory evaluation) for saltiness of the solution were assessed to understand the charge–charge interaction between CNFs and NaCl, the flow behavior of the CNF solution, and the ability of CNFs to enhance the saltiness.

2. Materials and methods

2.1. Materials

Squid (*Illex argentinus*) pens were donated as a gift from Shin Ho Sing Ocean Enterprise Co., Ltd. (Kaohsiung, Taiwan). Sodium acetate, Triton X-100, KOH, KBr, and $CO(NH_2)_2$ were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). NaCl was purchased from Uniregion bio-tech (Hsinchu, Taiwan).

2.2. CNF preparation

First, we rinsed the squid pen with pure water to remove impurities and then dried it in an oven at 50 °C for 3 days. Next, we ground it using a grinder (Yu-Chi Machinery, DV3-10, Changhua, Taiwan) and placed the powder in a sieve to obtain size of 125–150 μm squid pen powder.

We placed 50 g of squid pen powder in 500 mL of 5 g/L Triton X-100 and stirred the mixture at room temperature for 24 h to remove lipoprotein. Next, we rinsed the mixture with distilled water and placed it in 500 mL of 1 mol/L NaOH before stirring the mixture at room temperature for another 24 h to remove all protein residues. Subsequently, we rinsed the mixture with distilled water until neutral to obtain β-chitin. Because dried chitin fibers have strong hydrogen bonds, purified chitin must be kept moist to allow subsequent nanofibrosis (Lu et al., 2013; Nata, Wang, Wu, & Lee, 2012).

The water content of the wet chitin was soaked up by using a paper towel to ensure no water residue remained. Next, 0.5 g of the mixture was evaluated using an infrared moisture analyzer (Denver IR-35, Göttingen, Germany) to determine the water content, which was measured to be 20.8%.

The 0.36 g of purified chitin was used to make 0.5 g/L 150 mL aqueous chitin solution. The aqueous solution was placed in an ice bath and ultrasonicated at 20 kHz, 200 W and amplitude of 40% by using a vibra-cell ultrasonic processor (ChromTech UP-500, Apple Valley, Minnesota, USA). The ultrasonication times were 30, 45, and 60 min. The ultrasonication process involved cycles of 9 s of ultrasonication followed by a pause of 4 s. Subsequently, the aqueous solution was centrifuged at 12,000 rpm for 20 min to produce CNF suspensions under various ultrasonication conditions (Lu et al., 2013). The CNF suspensions were named CNF30, CNF45, and CNF60 to reflect the ultrasonication times of 30, 45, and 60 min, respectively.

Next, 15 mL of the CNF30, CNF45, and CNF60 suspensions were dried using a lyophilizer (Labconco 7754010, Kansas City, Missouri, USA). The dried CNFs were weighed to calculate their concentrations by using the following formula:

$$\text{Concentration of CNF} = \text{CNF weight after lyophilization}/\text{solution volume}$$

2.3. Functional groups and degree of deacetylation

The functional groups and degree of deacetylation (DD) of the CNFs were determined through Fourier transform infrared spectroscopy (FTIR) by adapting the method proposed by Baxter, Dillon and Anthony (1992). The lyophilized CNF powder was mixed with KBr at a ratio of 1:100. The mixture was dried at 60 °C for 3 days to prevent the –OH group from interfering with the FTIR measurements, and was then pressed into pellet form. The absorbance of amide 1 (1655 cm^{-1}) and the hydroxyl band (3450 cm^{-1}) was measured using an FTIR spectrometer (Bio-Rad FTS-155, Hercules, California, USA). The hydroxyl group band at 3450 cm^{-1} was utilized as an internal standard for calibrating the disc thickness and adjusting the chitin concentration. Triplicate measurements were averaged and applied to calculate the DD by using the following equation:

$$DD(\%) = 100 - (A_{1655}/A_{3450}) \times 115$$

where A_{1655} and A_{3450} denote the absorbance at 1650 and 3450 cm^{-1}, respectively.

2.4. Transmission electron microscopy

CNF shapes and diameters were observed using a transmission electron microscope (TEM; Hitachi H-7650, Tokyo, Japan). We irradiated high-energy electrons on the solid sample surfaces to generate signals such as penetrating electrons, reflection electrons, secondary electrons, and X-rays; these signals were then processed using the appropriate device to obtain the structural patterns of the solid samples. The CNF suspensions were diluted 10-fold by using reverse osmosis water and poured on a TEM copper mesh. After allowing time for the suspensions to adhere to the copper mesh, excess suspensions were removed using filter paper and air-dried. Next, TEM scanning was performed to observe the shapes and diameters of CNF. Finally, 20 nanofibers were randomly selected for calculating the diameter (Pereira et al., 2014).

2.5. Zeta potential

CNF solutions (concentration: 0.30 g/L) produced under various