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Enhancing saltiness perception using chitin nanofibers when curing tilapia fillets

Chih-Yuan Hsueh [a], Min-Lang Tsai [a, *], Tristan Liu [a, b, **]

[a] Department of Food Science, National Taiwan Ocean University, 2 Pei-Ning Road, Keelung 20224, Taiwan, ROC
[b] Institute of Research and Development, Standard Investment (China), West Tower No.2, Hongxia Industry Area, Lane 2759, Wan Yuan Road, 201103, Shanghai, China

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ABSTRACT

This study applied an ultrasonication method to prepare chitin nanofibers (CNFs) with a mean diameter of 111.6 nm, which were then used in a curing solution to increase the saltiness perception of cured tilapia fillets. Subsequently, various concentrations of citric, malic, or lactic acid were used to further enhance the fillets' perceived saltiness. The fillets cured with 0.12–0.18 g/L CNFs had significantly higher saltiness perception scores than the fillets cured with the control solution. The optimal perceived saltiness occurred when the 0.15 g/L group (CNF15) solution was added. In addition, the astringency perception of each fillet group differed insignificantly from the control. When CNF15 was prepared in 40 g/L NaCl curing solution, the addition of 3 g/L citric acid or 4 g/L malic acid, significantly increased the saltiness perception of the fillet and the tasters did not perceive any additional sourness. In contrast, further increasing the lactic acid concentration decreased the perceived saltiness of the fillet. Overall, in specific concentrations, CNFs can increase the saltiness perception of cured fish fillets. Perceived saltiness can be further enhanced by adding adequate amounts of citric or malic acid.

1. Introduction

In Europe, cured fish fillets account for a substantial proportion of commercial fish products and approximately 90% of these are cold marinades. In general, fish fillets are cured by soaking them in solutions containing table salt (NaCl) and organic acids (Szymczak, Szymczak, Koronkiewicz, Felisiak, & Bednarek, 2013), hence, such products usually have a high sodium content. The daily sodium intake suggested by the World Health Organization (WHO) is < 5 g, and an excessive intake of sodium is known to increase the risk of cardiovascular disease. Nonetheless, sodium intake in the United States, the United Kingdom, and various Asian countries have been previously reported as 8.2–9.4, 9.4, and 12.0 g/day, respectively, far exceeding the WHO suggested limit (Liem, Miremadi, & Keast, 2011). Therefore, reducing the amount of salt in food has become imperative. The saltiness perception induced by NaCl mainly comes from the sodium ions (Na^+). Hence, decreasing the sodium content of food decreases its flavor and leads to reduced customer acceptance. Accordingly, how to reduce the sodium content in food while maintaining customer acceptance is currently a critical topic in the food industry (Lawrence, Salles, Septier, Busch, & Thomas-Danguin, 2009).

Methods to reduce the sodium in food are categorized into chemical mechanisms, cognitive mechanisms, and product structure design (Busch, Yong, & Goh, 2013). Chemical mechanisms involve using salt substitutes (e.g., KCl, $CaCl_2$, and $MgSO_4$) and flavor additives, such as citric acid, spices, or sodium glutamate (Liem et al., 2011). Cognitive mechanisms refer to the gradual reduction of table salt use in the diet over a long period (Girgis et al., 2003). Initiatives have been implemented to increase consumer awareness of sodium reduction (Webster, Dunford, Hawkes, & Neal, 2011), and regulatory policies have forced manufacturers to label a food's sodium content (Pietinen, Valsta, Hirvonen, & Sinkko, 2007). An example of product structure design is the modification of the size, shape, and morphology of table salt grains, which can enable the table salt to quickly dissolve from its crystal form (Moncada et al., 2015; Rodrigues, de Souza, Mendes, Nunes, & Pinheiro, 2016). Previously, an inhomogeneous distribution of salt was reported to provide taste contrasts and reduce adaptation (Noort, Bult, Stieger,

* Corresponding author.
** Corresponding author.
E-mail addresses: tml@mail.ntou.edu.tw (M.-L. Tsai), Tristanliu@sfworldwide.com (T. Liu).

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& Hamer, 2010), while structuring agents affected the physico-chemical nature of food systems, such as their polyelectrolytic hydrocolloids and osmolality (Jiang, Tsai, & Liu, 2017; Mosca, Andriot, Guichard, & Salles, 2015).

Chitin is one of the most abundant biopolymers in nature, particularly, in arthropod exoskeletons, squid pen, and fungus (and yeast) cell walls. Its monomer is 2-acetamido-2-deoxy-β-D-glucose (*N*-acetyl-D-glucosamine), which forms a polysaccharide linked by β-1,4 glycosidic bonds. Chitin is minutely considered as a functional and/or biomedical materials owing to excellent properties such as non-toxicity, biocompatibility, biodegradability, environmentally friendly, mucoadhesion, hemostatic, adsorption properties, and so forth (Ding et al., 2015; Hsu, Chen, Chen, Tsai, & Chen, 2015; Philibert, Lee, & Fabien, 2017; Tan, Chin, Tsai, & Liu, 2015). However, Chitin cannot be dissolved or dispersed in water that limited its potential utilization (Nikoo, Benjakul, & Rahmanifarah, 2016), but preparing chitin as Chitin nanofibers (CNFs) facilitates its dispersal in water to create a colloid solution.

CNFs are produced by first obtaining chitin through the removal of proteins and minerals from the squid pens, shrimp or crab shells, or fungi, followed by the use of a physical, chemical, or electro-spinning method to draw nanoscale fibers from the collected chitin (Ding, Deng, Du, Shi, & Wang, 2014; Fan, Saito, & Isogai, 2008; Ifuku & Saimoto, 2012; Ifuku, Nomura, Morimoto, & Saimoto, 2011). CNFs have excellent properties, such as a nano-sized structure, very high surface-to-volume ratio, high water dispersibility and viscosity, high stiffness, low density, a high aspect ratio, high Young's modulus and a low thermal expansion (Ifuku et al., 2011; Mushi, Kochumalayil, Cervin, Zhou, & Berglund, 2016). Consequently, CNFs have considerable potential for filtration, biosensors and diagnosis, antibacterial application, wound dressing, tissue engineering and drug delivery, for instance (Ding et al., 2014; Jayakumar, Prabaharan, Nair, & Tamura, 2010; Philibert et al., 2017).

At pH < 7, the amine groups on the CNF molecular chains are protonated and have a positive charge (Fan, Fukuzumi, Saito, & Isogai, 2012; Fan et al., 2008; Jiang et al., 2017; Pereira, Muniz, & Hsieh, 2014). Therefore, CNFs can be used to adsorb dissociated chloride ions (Cl^-) in a NaCl solution and to form an electric double layer, thereby increasing the concentration of free Na^+ in the solution. This increases the likelihood that Na^+ will come into contact with taste receptors on the taste buds and, thus, enhances saltiness perception (Jiang et al., 2017).

In this study, the principle that the anions (Cl^-) are adsorbed with protonated CNF and higher content of free Na^+ in the solution is applied. The prepared CNFs with ultrasonication were used in a curing solution to increase the saltiness perception of cured tilapia fillets and to develop reduced-salt food products. CNF solutions of various concentrations were prepared by ultrasonication and were then mixed with a table salt solution to create curing solutions for fish fillets. The concentration ratio that optimized the saltiness perception was identified. Additionally, three types of edible organic acids (i.e., citric, malic, and lactic acids) were, respectively, added to the curing solutions to determine which acid most effectively increased the saltiness perception of the resulting tilapia fillet, without inducing a notably sour flavor.

2. Materials and methods

2.1. Materials

Squid (*Illex argentinus*) pens were donated by Shin Ho Sing Ocean Enterprise Co., Ltd. (Kaohsiung, Taiwan). Tilapia, which makes a considerable economic contribution in Taiwan, was selected for the experiments because of its low-cost, year-round availability, and simple processing. Raw tilapia fillets were purchased from a supermarket in Keelung. In addition, sodium acetate, Triton X-100, KOH, KBr, $CO(NH_2)_2$, citric acid, malic acid, and lactic acid were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). NaCl was purchased from UniRegion Bio-Tech (Hsinchu, Taiwan).

2.2. CNF preparation

The squid pen was washed with clean water to remove residues, dried in an oven, ground with a grinder, and then sieved to obtain squid pen powder (125–150 μm). Next, 30 g of powder was mixed and stirred with 300 mL of 5 g/L Triton X-100 at room temperature for 24 h to remove lipoproteins. The product was then washed with distilled water and stirred and mixed with 300 mL of 1 mol/L NaOH at room temperature for 24 h to remove residual proteins. Finally, the product was washed with distilled water until it became neutral, to obtain β-chitin. Producing fibers from dried chitin generates strong hydrogen bonds. Hence, purified chitin should be kept moist to facilitate subsequent treatment (Lu et al., 2013). Paper towels were used to dry the prepared moist chitin (until no further water could be absorbed). Next, 0.5 g β-chitin was placed in an infrared moisture analyzer (IR-35, Denver Instrument, Germany), which determined its water content to be 159 g/kg.

The purified chitin was used to make 150 mL of a 0.5 g/L aqueous chitin solution. Then, the chitin solution was placed in an ice bath and ground using an ultrasound cell grinder (Vibra-Cell Ultrasonic Processor, UP-500, ChromTech, Apple Valley, Minnesota, USA) at 20 kHz, 200 W, and 40% amplitude for 45 min, after which it was centrifuged at 12000 rpm for 20 min to obtain a CNF suspension (Lu et al., 2013). Subsequently, 10 mL of the suspension was freeze-dried (Labconco, Missouri, USA) and weighed to calculate its yield and concentration using the following equation:

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

2.3. Functional groups and degree of deacetylation (DD)

The functional groups and DD of the CNFs were determined using an infrared spectroscopy method (Tan et al., 2015). The lyophilized CNF powder was mixed with KBr at a 1:100 ratio. The mixture was dried at 60 °C for 3 days to prevent the interference of -OH groups in the spectroscopy measurements, after which it was pressed into pellet form. The absorbance of the amide I (1655 cm^{-1}) and hydroxyl bands (3450 cm^{-1}) was measured using Fourier transform infrared spectroscopy (FTIR; Bio-Rad FTS-155, Hercules, CA, USA). The band corresponding to the hydroxyl groups at 3450 cm^{-1} was used as an internal standard to calibrate the disc thickness and chitin concentration. Triplicate measurements were averaged and used to calculate the DD using the following equation:

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

where A_{1655} and A_{3450} are the absorbances at 1655 cm^{-1} and 3450 cm^{-1}, respectively.

2.4. Morphology

A freeze-dried CNF sample (5 × 5 mm, ~0.02 mg) was glued using carbon adhesive onto an aluminum platform. A gold-plating machine (ion-sputter, Hitachi E-1010; Tokyo, Japan) was used to plate 2-nm thick platinum films on the samples. The morphologies of the samples were observed by scanning electron microscopy