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Preparation of extra-small nisin nanoparticles for enhanced antibacterial activity after autoclave treatment

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ABSTRACT

Nisin is applied broadly in the food industry as an antimicrobial peptide. The objective of this study is to prepare nisin nanoparticles using free nisin by a facile nanoprecipitation technique and to investigate their antimicrobial activity after high-temperature processing. Transmission electron microscopic images showed that the size of extra-small nisin nanoparticles with different initial concentrations of nisin (0.1%, 0.3% and 0.5%) was 5, 10 and 15 nm, respectively. The nisin nanoparticles were stable at pH 5.0 with the smallest size. Moreover, nisin nanoparticles exhibited a higher antimicrobial activity than free nisin at a concentration below 2.0 mg/ml after autoclave treatment. These results suggested that nisin nanoparticles could serve as a potential food preservative.

1. Introduction

Although synthetic preservatives have potential toxicity to humans, they are still currently widely used in the food industry, especially in developing countries. Natural preservatives, such as natural active ingredients, essential oils and antimicrobial peptides, have attracted much attention because of their biocompatible and non-toxic characteristics (Reis, Paula, Casarotti, & Penna, 2012). Antimicrobial peptides show many advantages and prospects for widespread use in the food industry because of their low molecular weight, excellent water solubility and no side effects in humans (Yeom et al., 2016; Yu, Lo et al., 2017). In the food industry, only a few peptides are permitted to be used as food preservatives. Nisin is one of these few, and it is derived from a type of food-grade lactic acid bacterium (Kuipers et al., 1992). It has been approved by the US Food and Drug Administration for actual use in a number of foods because of its high efficiency and it has no toxic side effects (Saraniya & Jeevaratnam, 2015). However, the challenge for nisin to exert antimicrobial performance is that it must be dissolved and exhibit its activity in low pH levels (Prombutara, Kulwatthanasal, Supaka, Sramala, & Chareonpornwattana, 2012). Moreover, it loses part of its activity at a high temperature, especially when the high temperature is combined with low pH levels.

To date, many methods have been described to improve the biological activity of nisin. For example, liposomal delivery systems are designed to encapsulate nisin (Benech, Kheadr, Lacroix, & Fliss, 2002; Lopes, Pinilla, & Brandelli, 2017). However, this technique has some drawbacks, such as using an organic solvent in the preparation, high cost of phospholipids and uncontrolled dispersibility in size (Krivorotova et al., 2016). Alternatively, nanoparticles formed by food-grade biopolymers are used for encapsulating, protecting and delivering nisin. Recently, nanoparticle systems for delivering nisin based on alginate (Maresca et al., 2016), chitosan (Hu et al., 2017), chitosan-carageenan (Chopra, Kaur, Bernela, & Thakur, 2014) and chitosan/poly-g-glutamic acid (g-PGA) (Wu et al., 2016) have been developed, but these methods are complicated. Therefore, developing a simple method of preparation for nisin nanoparticles is urgently needed in the food industry.

The objective of this study is to prepare nisin nanoparticles using free nisin by a facile nanoprecipitation technique and to investigate their antimicrobial activity against *Staphylococcus aureus* after high-temperature processing. The effects of initial nisin concentration and pH values on the mean particle size of nisin nanoparticles were evaluated. In addition, the characteristics of as-prepared nisin nanoparticles were measured by X-ray diffraction (XRD) and Fourier transform infrared (FTIR) spectroscopy analysis.

2. Materials and methods

2.1. Materials

Nisin was obtained from Sinopharm Chemical Reagent Limited Company (Beijing, China). *S. aureus* was supplied by Shanghai Luwe Technology Limited Company. All other reagents used were of analytical grade.

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2.2. Preparation of nisin nanoparticles

To prepare the nisin nanoparticles by a nanoprecipitation method (Yu, Lu et al., 2017), the required amount of nisin (1, 3 and 5 mg/ml) was fully dissolved in a hydrochloric acid aqueous solution (100 ml). A certain amount of distilled water (50 ml) was added dropwise to the nisin solution with different pH values (0.9, 1.7, 3.0, 5.0 and 7.0). The dispersion was constantly stirred at 25 °C for 5 h. The as-prepared nisin nanoparticles were freeze-dried. To examine the various pH values and temperatures in the antibacterial activity, the nisin nanoparticle powder was dispersed in an aqueous solution with pH values at 1.0, 3.0, 5.0, 7.0, 9.0, 11.0 and 13.0 at ambient temperature. The nisin nanoparticle dispersion (pH 5.0) was heated at 25 °C, 50 °C, 100 °C and 121 °C for 30 min.

2.3. Dynamic light scattering (DLS)

The mean size, polydispersity index (PDI), and ζ-potential of the samples were determined by DLS using a Malvern Zetasizer Nano (Malvern Instruments Ltd., UK). The samples were diluted in MilliQ water and analysed at 25 °C. The concentration of diluted samples was 0.05%.

2.4. Transmission electron microscopy (TEM)

The TEM images of nisin nanoparticles were observed on a 7700 transmission electron microscope (Hitachi, Tokyo, Japan). A tiny drop of the sample was deposited on a carbon-coated copper grid and then freeze-dried for observation.

2.5. FTIR spectroscopy

The chemical structures of the free nisin and the nisin nanoparticle powders were confirmed using FTIR spectra (Jasco Inc., Easton, MD, USA). The samples were ground together with potassium bromide and pressed into disks for scanning. A total of 32 scans with a 4 cm^{-1} resolution were accumulated by the rapid-scan software in OMNIC 8.0 to obtain a single spectrum.

2.6. XRD

XRD was conducted using an X-ray diffractometer at 40 kV and 25 mA. The diffracted beam was collected with a 2D GADDS detector. The distance from the sample to the detector was 100 mm. The scanning range of the diffractograms was 4–90° (2θ) with a rate of 1.0/min.

2.7. UV–vis absorption spectrum

The free nisin and nisin nanoparticles at a concentration of 0.5% were dispersed in a water solution (pH = 1.7 and 5.0). The absorption spectrum of the samples was scanned at a wavelength of 190–900 nm at 1 nm intervals to obtain a spectrum.

2.8. Antibacterial analysis

Antibacterial activities of the free nisin and the nisin nanoparticles solution (pH = 5.0) with different concentrations autoclaved at 121 °C for 20 min (Davies et al., 1998) were examined as the inhibitory effects against the growth of Gram-positive bacteria *S. aureus*. The growth of bacteria was monitored by taking OD at 600 nm according to the previous report with some modification (Bernela, Kaur, Chopra, & Thakur, 2014; Carson, Mee, & Riley, 2002). The strains were aseptically inoculated in broth medium and subsequently incubated at 37 °C for 12 h. An inoculum (0.5 ml) of *S. aureus* were aseptically transferred to 1 ml of broth containing the free nisin or nisin nanoparticles samples with different concentrations and subsequently incubated at 37 °C for 8 h under mild shaking. Culture with an untreated nisin solution was used as the control. The inhibitory effect was estimated by measuring the turbidity of the cultured medium at 600 nm using a spectrophotometer.

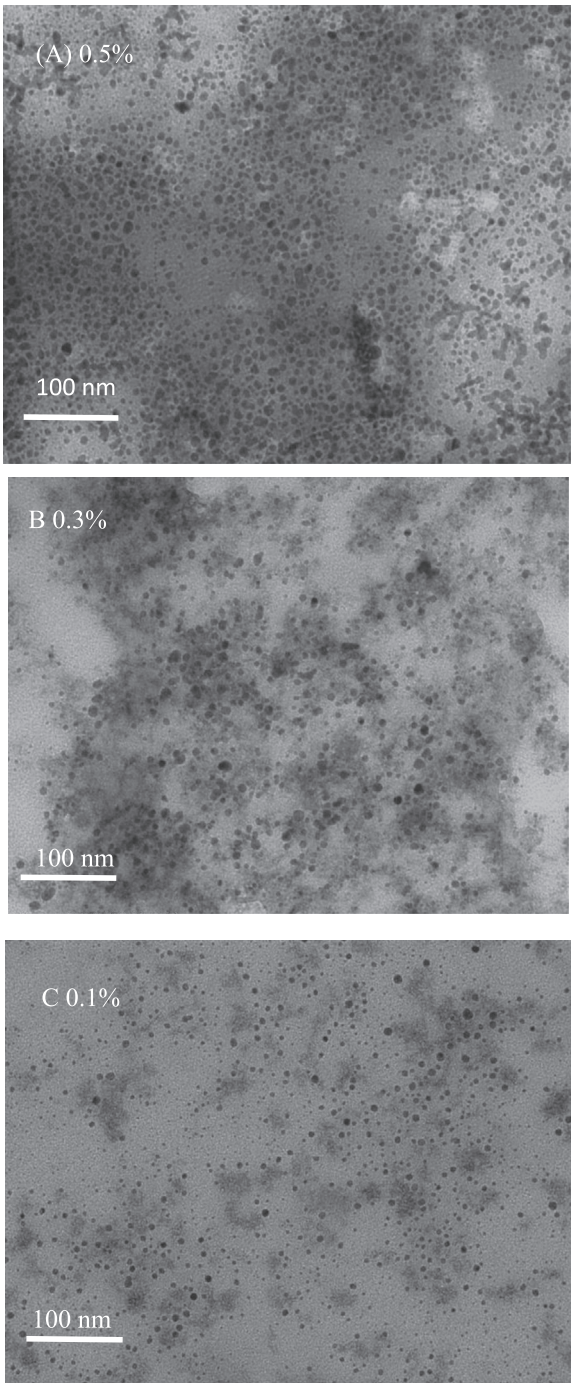

Fig. 1. TEM images of the nisin nanoparticles with different initial concentrations of free nisin (A 0.5%, B 0.3%, and C 0.1%) at pH 1.7.

2.9. Statistical analysis

All tests were conducted at least in triplicate. The results were reported as the average values and standard deviations. The data were analysed by ANOVA using the SPSS V.17 statistical software package (SPSS Inc., Chicago, IL).