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Formation and optimization of chitosan-nisin microcapsules and its characterization for antibacterial activity

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ABSTRACT

Nisin, a natural antimicrobial agent, was selected as the core material with chitosan (CS) as the wall material for micro-capsulation. Response surface methodology was used to optimize the process of preparing CS–nisin microcapsules. The predicted optimal condition was found to be CS concentration of 2.4 mg/ml, salt addition rate of 8 ml/min, CS: nisin by 3.8:1 (W/W) ratio, Na_2SO_4 as the precipitant salt. In this condition, the embedding, release rate, Z-average size of the microcapsules were 68.7%, 54.6%, 1441.6 nm, respectively. According to the predicted model, the corresponding values were 66.9%, 50.8%, 1419 nm, respectively. Scanning electron microscope (SEM) revealed that CS-nisin microcapsules were spherical, and the size apparently increased compared with CS. Fourier transform infrared spectroscopy (FTIR) indicated that there was no significant chemical interaction between CS and nisin. Antimicrobial activity assay exhibited CS-nisin microcapsules with the concentration of 0.25% (W/V) showed confirmed inhibitory effect against Gram-positive bacteria *Bacillus subtilis*. Besides, 1% CS-nisin showed the maximum antibacterial activity at pH 5.0–6.0, when the value of pH was 7.4, it also exert antibacterial ability, indicating the enhanced antimicrobial activity of CS-nisin.

1. Introduction

Antimicrobial peptides (AMPs), composed of 20–60 amino acids, are small peptides playing active roles in the resistance of bacteria, fungi, protozoa, suppression of tumor cell killing et al. encoded by a variety of biological cell-specific genes, which are induced by external conditions (Keymanesh, Soltani, & Soroush, 2009). Because of their low molecular weight, high heat-tolerant, excellent water-soluble, no immunogenicity and no adverse effects on human, AMPs show great advantages and broad application prospects in the food industry. Among all the antimicrobial peptides, only a very few of them are actually allowed to be used as preservative in the food industry. Nisin is one of these few, it is a kind of bacterial antimicrobial peptide produced by a food grade lactic acid bacterium named Lactococcus lactis subsp. Lactis (Breukink & Kruijff, 1999). It exhibited a broad antimicrobial spectrum against Gram-positive bacteria. Besides, nisin obtained national standard allows for practical application in some foods for its high-efficient and non-poisonous nature (Juneja, Dwivedi, & Yan, 2012). However, nisin can interact with some food components such as proteins, lipids and pro-metabolic enzymes, which lead to the loss of its biological activity. Moreover, it can only play an active role in low pHs (Prombutara, Kulwatthanasal, Supaka, Sramala, & Chareonpornwattana, 2012).

To improve the quality of nisin, many methods were developed. Liposomal delivery systems were proposed to encapsule nisin (Colas et al., 2007; Imran et al., 2014; Laridi et al., 2003; Malheiros, Sant'Anna, Barbosa, & Brandelli, 2012). But this method has many drawbacks, such as the usage of organic solvent in traditional liposomes preparation methods is adverse to safety; uncontrolled polydispersity in size, high cost of phospholipids.(Krivorotova et al., 2016). There are alternative ways using foodgrade biopolymers for encapsulating, protecting and delivery nisin. Delivery systems for nisin based on alginate (Maresca et al., 2016), chitosan-carageenan (Chopra, Kaur, Bernela, & Thakur, 2014), chitosan/alginate (Zohri et al., 2010) were well developed.

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Chitosan (CS) is a natural cationic polymer derived from the deacetylation of chitin, consisted of copolymers of glucosamine and N-acetyl-glucosamine. Own to its remarkable versatility and unique characteristics, it is widely used for various purposes, such as solutions, gels, films and fibers (Rinaudo, 2006). Moreover, the advantages of biodegradability, low toxicity and good biocompatibility make it a great potential for biomedical and pharmaceutical applications. Up to now, CS has been reported as carries for drugs, proteins, vaccines, enzymes (Kamburov & Lalov, 2014; Ko, Park, Hwang, Park, & Lee, 2002; Sanyakamdhorn, Agudelo, & Tajmir-Riahi, 2013; Sinha et al., 2004) and materials for preparing hybrid nano-composite particles (Liu et al., 2011). Many methods were reported to prepare chitosan based microcapsules, including emulsion-precipitation, crosslinking, emulsion-precipitation, precipitation-coacervation. However, the first two methods were found to have adverse effects when encapsulating labile proteins like nisin. The use of crosslinking agent could even induce safety concerns (Huang, Yeh, & Chiang, 2002; Lubben, Verhoef, Aelst, Borchard, & Junginger, 2001). Both emulsion-precipitation and precipitation-coacervation can applied in preparation of CS particles in mild conditions, while the latter is preferred because of the higher drug/protein loading efficiencies (Gan & Wang, 2007; Özbaş-Turan, Akbuğa, & Aral, 2002). Koppolu et al. (2014) applied CS for the delivery of proteins and vaccines by precipitation-coacervation method and the effects of CS concentration, CS molecular weight, precipitant salt composition, precipitant salt addition rate, sonication power, and protein size on the efficiency of particle size, polydispersity and protein loading efficiencies were studied. Another study use CS-tripolyphosphate nanoparticles for the delivery of tea catechins, by investigating the effects of CS molecular mass, CS concentration, CS-TPP mass ratio, initial pH value of CS solution, and concentration of tea catechins on encapsulation efficiency and the release profile of tea catechins in vitro (Hu et al., 2008). Delivery systems based on nano/micro chitosan have been reported for many times (Bernela, Kaur, Chopra, & Thakur, 2014; Jeevitha & Amarnath, 2012; Yu et al., 2014), however, no defined model for production and optimization of CS–nisin microcapsule was reported yet. It is well known there are alternative ways to optimize the production process of microcapsules, such as the use of response surface methodology.

Response surface methodology (RSM) is a statistical method with reasonable methods and experimental design experiments to obtain certain data, multivariate quadratic regression equation to fit a function of factors and response between the values, making out the optimal process parameters analysis through the regression equation. RSM is a good tool for evaluation, preparation, optimization (Esmaeili & Gholami, 2015).

The aim of this study is to prepare and optimize CS-nisin microcapsules by precipitation-coacervation technique with RSM, which not only protect nisin from the food environment and prolong its biological activity but also get a new natural, non-toxic, affordable material to meet the demand for preservation in food industry.

2. Materials and methods

2.1. Materials

Chitosan (CS, Mw 50000-100000, degree of acetylation 85%) were purchased from Qingdao Yun Yu Biotechnology Co., Ltd., nisin (activity of 10^6 IU/g) was obtained from Biological Engineering Co., Ltd. Zhejiang Yinxiang. Tween 80, acetic acid, sodium sulfate and trisodium citrate dihydrate were of analytical grade, purchased from Sinopharm Chemical Reagent Co., Ltd.; *Bacillus subtilis* CICC10275 was obtained from China center of industrial culture collection.

2.2. Production of chitosan –nisin microcapsules

CS (0.1%–0.5%, W/V) was dissolved in 1% acetic acid by ultrasonic for 10 min till its ultimate dissolution. Nisin stock solution was prepared by diluting the stock powder in 1% acetic acid (with the ratio of CS: nisin = 1:1, 3: 1, 5:1, W/W) was added with stirring. CS-nisin particles were formed by adding 50 mM precipitant salt solution (sodium sulfate or trisodium citrate dihydrate) drop wise to the CS solution using a peristaltic pump (BT100-2J, Hebei Lange current pump Ltd, China) and Tween 80 (1.5%, V/V) was added as a nonionic stabilizer. The suspension was obtained after stirring 2–4 h. CS-nisin particles were collected after centrifugation at 7,155 g for 10 min at 4 °C and vacuum freezing dried with a freeze dryer (Labconco, Beijing Light Ace HK Limited, China) for 2 days under −80 °C for further use. Wherein, CS concentration, salt addition rate, the adding rate of CS and nisin were changed in various values to optimize CS–nisin microcapsules.

2.3. Size and polydispersity index (PDI)

Size and polydispersity index (PDI) of the resultant particles were determined by the size and zeta potential analyzer (Zetasizer Nano ZS90, UK). 10 mg CS-nisin was dissolved in 1000 mL 1% acetic acid by ultrasonic for 10 min till ultimate dissolution for further use.

2.4. Assay of nisin embedding rate

The embedding rate was determined by the concentration of nisin remained in the supernatant after removing the nisin-loaded chitosan particles. The concentration of nisin was determined by HPLC, using a C-18 reverse-phase column (ZORBAX 300SB-C18, 4.6 × 250 mm, Agilent, USA) eluted with 10% acetonitrile/0.1% (v/v) TFA (eluent A) and 90% acetonitrile/0.05% (v/v) TFA (eluent B) (gradient: 0–8 min, 15% eluent B; 8–35 min from 15% eluent B, to 55% eluent B; 55–60 min, 15% eluent B) with UV detection at 220 nm. The amount of nisin was calculated by means of a calibration curve, derived from standards of nisin. And then the embedding rate was calculated indirectly with eq. (1)

$$\text{Embedding rate} = \frac{(\text{Initial conc.} - \text{Unencapsulated conc.})}{\text{Initial conc.}} \times 100\% \quad (1)$$

2.5. Assay of nisin release rate in vitro

The nisin release rate was performed by suspending 50 mg CS-nisin particles into 1 mL of 0.02 M phosphate buffer (pH 5.0). The suspensions were stirred and maintained at room temperature under shaking at 150 rpm for 20 min. After standing for some time, the supernatant was filtered with a membrane. The nisin content in the buffer was determined by reverse-phase HPLC, as previously described. All samples were tested in triplicate.

2.6. Characterization of the CS-nisin particles

The surface micrographs of the CS-nisin particles were imaged by scanning electron microscopy (SEM) (SU-8010, Japan). Infrared Spectroscopy (IR) (Thermo Scientific Nicolet Avatar 370, USA) were carried out to confirm the information about chemical bond or functional groups through fourier transform infrared spectroscopy with the spectrum varying from 4000 to 400 cm^{-1}.