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Dynamic encapsulation of hydrophilic nisin in hydrophobic poly (lactic acid) particles with controlled morphology by a single emulsion process

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ABSTRACT

Hydrophilic nisin-loaded hydrophobic poly (lactic acid) (PLA) particles with controlled size and shape were successfully produced utilizing a one-step single emulsification method. Preliminary shear stress and temperature tests showed that there was no significant loss in the nisin inhibition activity during this process. PLA/nisin composite particles were prepared into solid nanocomposite spheres (50–200 nm) or hollow microcomposite spheres (1–5 μm) under the operative conditions developed in our previous study, in which the hydrophilic nisin in the aqueous phase solution could be entrapped in the hydrophobic polymer in the emulsification process generating either single or multiple emulsions. The incorporation of nisin in PLA had little effect on key processing conditions, which allows the dynamic control of the morphology and property of resulting particles. Microscopic and surface analyses suggested that nisin was dispersed uniformly inside the polymer matrix and adsorbed on the particle surface. The encapsulation amount and efficiency were enhanced with the increase in nisin loading in the aqueous solution. Unique reversible control of particle size and shape by this process was successfully applied in the nisin encapsulation. Alternating temperature in the repeating emulsification steps improved the encapsulation efficiency while generated particles in desired size and shape.

1. Introduction

The outbreaks of foodborne pathogenic bacteria from food and drinking water post-production have caused millions of infections, hospitalizations, and deaths in the United States each year [1–3]. With increasing public concerns on foodborne illnesses, there are great needs to develop new post-processing technologies to improve food safety and quality as well as extend product shelf-life [4].

Antimicrobial agents as preservatives can be added into food during preparation [5]. They can prevent the growth of present spoilage and pathogenic microorganisms from the post-production contamination, which could significantly reduce the excessive processing of food and further lower the production and transportation costs. The commonly used antimicrobial agents are chemicals including sodium chloride and organic acids, and natural antimicrobials such as essential oils, bacteriocins, etc. [6–10]. Natural antimicrobials are preferred for manufacturing because of customers' increasing concerns on chemical additions.

Nisin is one of the most widely studied bacteriocins obtained from a great number of Gram-positive and Gram-negative bacteria strains [10,11]. It has been approved by Food and Drug Administration (FDA) and World Health Organization (WHO) for the use as a generally recognized as safe (GRAS) food additive [12,13]. Nisin is a low molecular weight polypeptide composed of 34 amino acids. Generally, nisin has two variants A and Z, which differ in a single amino acid residue at position 27 where is histidine in nisin A and asparagine in nisin Z [12]. The amino acid sequence and proposed structure of nisin Z are given in Fig. 1 [14,15]. Similar to other peptides or proteins, there are both hydrophobic and hydrophilic amino acid units in nisin compositions. It is commercially produced by many strains of *Lactococcus lactis* and well known to be effective against a wide variety of Gram-positive bacteria. Its mode of action is considered to involve the interaction with phospholipids in the cytoplasmic membrane of bacteria cells and the formation of pores on the membrane, resulting in the rapid leakage of essential cytoplasmic components [16]. Different forms of nisin either alone or in combination with other compounds have been applied in controlling pathogens in food [17]. However, its antimicrobial efficacy can be rapidly decreased when interacted with food components, such as proteins, lipids, enzymes, etc., which

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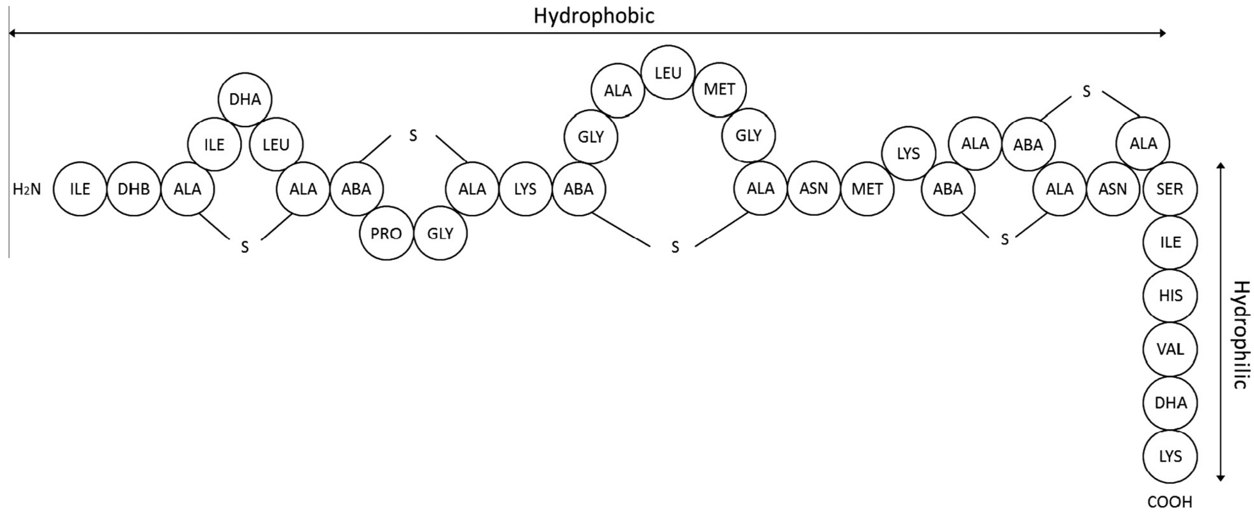

Fig. 1. The primary structure of nisin Z showing amino acid units in the hydrophilic and hydrophonic sides of the molecule (adapted from [14]).

leads to a lower accessibility to target bacteria [18]. Antimicrobial activity loss can also be induced by the digestion with proteolytic enzymes or inhomogeneous distribution in food systems [19]. Moreover, with the decrease in nisin activity, bacteria tend to regrow in food products [20]. Hence, excessive amount of nisin is required to achieve effective antimicrobial inhibition. To overcome these problems, different strategies have been investigated to enhance their stability and efficacy in inhibiting microbial growth.

Microencapsulation has been developed to encapsulate the actives into colloidal particles, on the purpose of protecting the actives from attacks in the surrounding environment or controlling their release rate into the medium [21]. This technology has been widely used as a delivery system to entrap and homogeneously disperse nisin in the food matrix, which not only protects nisin from unfavorable conditions but also provides the control release [22]. The incorporation of nisin in the matrix of calcium alginate to form microparticles provided the protection against proteolytic inactivation to bacteriocins or other protease-sensitive compounds [23]. Laridi et al. have reported the successful encapsulation of nisin Z in liposome vesicles and showed the stability of nisin-loaded vesicles in the Cheddar cheese temperature cycle without severe disturbance to cheese fermentation process [24]. The main limitation of incorporating nisin in liposomes is their interaction and thus the disruption of liposomal membranes. Moreover, the physico-chemical properties and release profile of nisin are strongly affected by environmental conditions, such as the bivalent ions and other medium components. Nisin-loaded poly-L-lactide (PLA) nanoparticles were also prepared by compressed CO_2 anti-solvent precipitation technique allowing a slow protein release and protein stabilization for long lasting antimicrobial activity [25]. The choice of the organic solvent is limited by different solubilities of protein and polymer, which may modify the protein structure by affecting the hydrophobic/hydrophilic interactions. The high pressure input and expensive process based on anti-solvent precipitation in supercritical CO_2 can be troublesome for large scale production. Xiao et al. used a spray drying process to encapsulate nisin in capsules with the presence of Tween 20, which showed the improvement on their antilisterial properties in milk [26]. The incorporation of nonionic surfactant facilitated to modulate the release kinetics of nisin. Although the spray drying offers a wide range of particle processing possibilities by altering process parameters, the exposure to heat and degradation of bioactive compounds are often noted to limit its applications in food and pharmaceutical industries.

The direct addition of nisin in the edible films or coatings has been increasingly studied in food packaging applications as a hurdle technology for food preservation [27]. Sebti et al. have incorporated nisin and stearic acid in hydroxyl propyl methyl cellulose (HPMC) based film by casting technology as a moisture barrier and antilisterial film on food surfaces [28]. However, film mechanical resistance and antimicrobial activity were reported to be reduced. The matrix used for antimicrobials is of great importance to support these active compounds as well as provide a slow release of them. Besides HPMC, PLA and its copolymer polyglycolic acid have been proposed as active packaging options. In addition, embedding nisin in biopolymers to form biodegradable antimicrobial packaging films was investigated to develop the controlled delivery of nisin while maintaining the appropriate protection for food. Jin and Zhang have prepared PLA/nisin film via a solvent casting method and showed its effective inhibition of *Listeria monocytogenes* growth in culture medium and liquid egg white, which pointed out the application of PLA/nisin polymer as the film or surface coating on solid or liquid food packaging [29].

Polymer systems have been extensively applied for the encapsulation and delivery of active compounds. Among different types of carriers, polymer particles are one of the most attractive strategies due to their controllable properties, flexible and reproducible production processing, etc. The control of polymer particle size and/or shape has been the subject of many studies, attributed to the effective combination of their unique features and improved properties [30–33] for the effective loading/encapsulation and delivery of active species [21]. The incorporation can be achieved by either the entrapment of active compounds in the polymer matrix or the adsorption of them onto the polymer particles. Owing to its biodegradable, non-toxic, and GRAS properties, PLA has been largely used in the sustained and controlled release system for food, pharmaceutical, and biomedical industries [34]. Nisin has been loaded in the PLA matrix via different approaches. Nevertheless, the fabrication was limited due to the intrinsic hydrophobicity of PLA giving rise to the poor interfacial adhesion between nisin and polymer matrix and thus affecting the embedding capacity of nisin.

The present study was to develop a PLA-based encapsulation system that could provide the uniform dispersion of hydrophilic nisin in the hydrophobic polymer matrix with controlled morphologies by a mild, efficient, and chemical-reduced process. The emulsion technique is one of the most promising methods to obtain versatile incorporation of hydrophilic or hydrophobic materials