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Protective effects of soybean protein and egg white protein on the antibacterial activity of nisin in the presence of trypsin

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ABSTRACT

The using of nisin to prevent foodborne pathogens (*Staphylococcus aureus* and *Listeria monocytogenes*) from contamination has received broad attentions during meat processing. However, the application of nisin has been limited because its antibacterial activity may be inhibited by trypsin. In this study, the protective effects of soybean protein and egg white protein on antibacterial activity of nisin were evaluated. It could be concluded that exogenous trypsin decreased the antibacterial activity of nisin, soybean protein and egg white protein could keep the nisin activity from enzymolysis of trypsin. Trypsin inhibitors in soybean protein and egg white protein could protect the antibacterial activity of nisin. Nisin with soybean protein or egg white protein in cooked meat product presented better quality preservation effects than nisin alone in the presence of trypsin. The total viable counts (TVC) and total volatile basic nitrogen (TVB-N) of nisin-treated group were significantly higher than these in nisin-soybean protein-treated and nisin-egg white protein-treated groups with trypsin. This study showed the potential of using soybean protein and egg white protein to stabilize the antibacterial activity of nisin under high trypsin conditions.

1. Introduction

As an important animal source food, meat products have been favoured for their good taste and high protein nutrient values (Givens, 2005). With the improvement of people's living standard, the demand for meat products is increasing in developing countries including China. However, bacterial contamination of meat products can pose threats to consumer health and lead to economic losses on the meat producer (Hui, Liu, Feng, Li, & Gao, 2016; Nychas, Skandamis, Tassou, & Koutsoumanis, 2008). Although chemical preservative is one of the most conventional choices for the preservation of meat products, there are some security issues, such as carcinogenicity for consumer, uncertain chemical pollutants in the food et al. (Bono, Badalucco, Cusumano, & Palmegiano, 2012; Larsson, Orsini, & Wolk, 2006). Consequently, the demand for natural preservatives is increasing (Bazargani-Gilani, Aliakbarlu, & Tajik, 2015; Economou, Pournis, Ntzimani, & Savvaidis, 2009).

Nisin is a natural bacteriocin produced by certain lactic acid bacteria (He et al., 2016). Due to its function on Gram-positive bacteria by forming pores in the target cell membrane (Bruno & Montville, 1993; De Arauz, Jozala, Mazzola, & Vessoni Penna, 2009; Ruhr & Sahl, 1985), nisin plays an important role in the field of food preservation, such as fresh chilled pork (Wang et al., 2016), milk (Kim, Choi, Bajpai, & Kang, 2008), fish meat et al.(Cabo, Herrera, Sampedro, & Pastoriza, 2010). In fact, nisin is the only bacteriocin approved to be used in the food field by the Joint Food and Agriculture Organisation and World Health Organisation (FAO/WHO) (Naidu, 2000).

However, the activity of nisin can be influenced by various factors, such as pH value in food, temperature of storage, enzyme, lipids of food components et al. (Aasen et al., 2003; Chollet, Sebti, Martial-Gros, & Degraeve, 2008). Different strategies have been developed to enhance the stability and prolong the efficacy of nisin in food system. Pectin nanoparticles have been used to deliver nisin and prevent nisin from the interaction with food components during food processing and storage period (Krivorotova et al., 2016). Carbohydrate carriers such as starch octenyl succinate in the emulsion systems could stabilize and retain nisin activity in a cantaloupe juice model (Sarkar, Bhunia, & Yao, 2017). Corn zein, as a carrier biopolymer of nisin, could enhance the antibacterial efficacy of nisin inhibiting the growth of *Listeria monocytogenes* (Xiao, Davidson, & Zhong, 2011). Liposomes have been used for

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the encapsulation of nisin and can stabilize its efficacy at 4 °C for 27 days in different media including milk, skim milk, sweet whey and phosphate buffer saline (PBS) (Laridi et al., 2003). However, these methods do not fundamentally solve the shortcoming of nisin's sensitivity to trypsin.

As a digestive enzyme, trypsin is widely distributed in meat products. It can inhibit the activity of nisin by enzymolysis (Slootweg, Liskamp, & Rijkers, 2013). Hence, it is important to inhibit the activity of trypsin for the stability of nisin. Soy protein is an important food-grade nutrition enhancer, and it contains a variety of anti-nutritional factors including trypsin inhibitors (Koide, Ikenaka, & Tsunasawa, 1973). Egg white protein is a kind of dried egg products, and it has a wide range of functions in the food industry. Ovomucoid in egg white protein is also a trypsin inhibitor of glycoprotein (Fraenkel-Conrat et al., 1949). However, the effect of trypsin inhibitors in the two proteins on nisin stability is little known in the presence of trypsin.

Therefore, the purpose of this study in its first phase was to determine the effects of soy protein and egg white protein on trypsin. Then we investigated the effects of the two proteins on antibacterial activity of nisin in the presence of trypsin. Finally, the protective effects were validated in the cooked pork.

2. Materials and methods

2.1. Chemicals

The chemicals including nisin (30%, 12,000 IU/mg), soybean protein (99%), and egg white protein (99%) were obtained from Zhengzhou Mindtek Biotechnology Co. Ltd, China. Trypsin (≥200,000 U/g) and other chemicals were purchased from Beijing Dingguo Changsheng Biotechnology Co. Ltd, Beijing, China. All chemicals used in the experiments were of analytical grade.

All solutions, such as soybean protein, egg white protein, trypsin, and nisin solution were prepared with HCl solution (pH 7.5 0.02 moL/L). They were stored at 4 °C before using.

2.2. Effects of the two proteins on trypsin activity

Different volumes of soybean protein or egg white protein solution were added to 1 mL trypsin solution (0.75 g/L). Each mixture was allowed to interact at 40 °C for 1 h. Following incubation, the trypsin activity was determined by the method of National Standard of People's Republic of China (GB/T23527-2009). The reaction solutions were mixed with 1 mL of substrate (10 g/L casein solution). The mixtures were incubated at 40 °C for 10 min, then the reactions were ended through the addition of 2 mL trichloroacetic acid (TCA, 65.4 g/L). Subsequently, the solutions were centrifuged at 1000g for 10 min. Then 1 mL of the supernatant was withdrawn and added into 5 mL of Na_2CO_3 solution (42.4 g/L), and 1 mL of the Folin-Ciocalteu phenol reagent (diluted with deionised water in the ratio of 1:2 v/v) at 40 °C for 20 min. The absorbance was measured at 680 nm with an ultraviolet visible spectrophotometer (UV-1700, Shimadzu, Japan). The trypsin activity was calculated using the following Eq. (1):

$$X(U/g) = \frac{A_1 \times V_1 \times 4 \times N}{m} \times \frac{1}{10} \quad (1)$$

where A_1 was the enzyme activity of the sample in the standard curve (U/mL), V_1 was the volume of the volumetric flask used to dissolve the sample (mL), N was the dilution factor of the sample, and m was the weight of trypsin (g).

2.3. Determination of nisin titer

To determine the titer of nisin in various preparations, agar diffusion assay with standard curve method was employed (Sarkar, Bhunia, & Yao, 2016). Four groups of working formulations were prepared: (1) Control group, in which both nisin and HCl solution (pH 7.5 0.02 moL/L) were mixed. (2) Experimental group 1, in which both nisin and different volumes of trypsin solution were mixed. (3) Experimental group 2, in which nisin, different volumes of trypsin and soybean protein were mixed. (4) Experimental group 3, in which nisin, different volumes of trypsin and egg white protein were mixed. The ratio of various substances was shown in Tables 1 and 2, respectively. All the mixtures were allowed to interact at 40 °C for 1 h. Agar diffusion assay with standard curve method was used to determine the nisin titer.

2.4. Validation experiment in cooked pork

2.4.1. Preparation of cooked pork samples

Fresh pork tenderloin (ca. 1500 g) samples with a uniform shape were obtained on November 5, 2016 from a local market in Zhengzhou, China, and they were kept at 0 °C for more than 1 h before using. The samples were sliced into uniform flakes (10 g), washed (by deionised water) and cooked in boiling-water for 20 min. The cooked pork samples were randomly divided into seven groups (3 samples in each stage of each group), as shown in Table 3. All samples were immersed in different solutions for 1 min and immediately packaged in food preservation. The cooked samples were stored at 4 °C and taken at determined time for the following evaluation.

2.4.2. Determination of TVC

A total of 10 g sample was chopped, and mixed for 2 min with 90 mL of 0.9% NaCl solution. The total viable counts (TVC) was determined by the National Standard of People's Republic of China (GB4789.2-2010). Briefly, for each sample, appropriate serial decimal dilutions were prepared in 0.9% NaCl solution. A total of 15–20 mL agar medium was transferred to sterile dish (90 mm × 90 mm) containing 1 mL of these serial dilutions of sample. The sample dilution and agar medium were adequately mixed. The TVC was determined using Plate Count Agar (PCA) after incubation at 37 °C for 48 h. The results were reported as CFU (colony forming units)/g.

2.4.3. Physicochemical examination

A total of 10 g sample was homogenized with 90 mL of deionised water (pH 7.0) and filtered after 30 min. The filtrate was used for the determination of total volatile basic nitrogen (TVB-N). The value of TVB-N was measured by the microscale diffusion method of the National Standard of People's Republic of China (GB/T5009.44-2003). Briefly, a total of 1 mL of H_3BO_3 (20 g/L) solution

Table 1
Effect of soybean protein on the titer of nisin.

Treatment	The titer of nisin (IU/mL)
Nisin	198.865 ± 0.450 a
Nisin:Trypsin (1:1)	168.461 ± 1.969 c
Nisin:Trypsin:Soybean protein (1:1:2)	199.144 ± 2.792 a
Nisin:Trypsin (1:2)	155.242 ± 2.181 c
Nisin:Trypsin:Soybean protein (1:2:4)	197.852 ± 3.123 a
Nisin:Trypsin (1:3)	139.759 ± 2.180 c
Nisin:Trypsin:Soybean protein (1:3:6)	200.694 ± 2.071 a

Soybean protein and trypsin were mixed with nisin in varying mass ratios. The mixtures were allowed to interact at 40 °C for 1 h. Agar diffusion assay with standard curve method was used to determine the nisin titer.
Different letters in the same column indicate significant differences ($P < 0.05$).