Anti-listeria effects of chitosan-coated nisin-silica liposome on Cheddar cheese

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ABSTRACT

Listeria monocytogenes poses an increasing challenge to cheese production. To minimize the risk of bacterial contamination, a chitosan-coated nisin-silica liposome was engineered for the present study. We investigated the characteristics of nisin-silica liposomes and the anti-listeria effects of a chitosan-coated nisin-silica liposome on Cheddar cheese. The encapsulation efficiency of nisin in a liposome was sharply increased after it was adsorbed on a silica particle surface. Chitosan-coated nisin-silica liposomes displayed sustained antibacterial activity against *L. monocytogenes*, without affecting the sensory properties of the cheese. Chitosan-coated nisin-silica liposomes could be a promising active antimicrobial for cheese preservation.

Key words: nisin-silica liposome, chitosan coating, *Listeria monocytogenes*, Cheddar cheese

INTRODUCTION

Cheese is a popular dairy product due to its inherent high nutritional value, flavors, and textures. However, if cheese products are contaminated by pathogenic bacteria, bacteria multiply rapidly under eutrophic conditions. In the United States, 65 foodborne outbreaks linked to cheese were reported between 1993 and 2006 (Yoon et al., 2016). *Listeria monocytogenes*, a notable species of gram-positive bacteria, is frequently detected in cheese and can cause serious foodborne illness (Lin et al., 2016). It has a fatality rate of 30%, possibly higher for people with low immunity and pregnant women (Chen et al., 2016). The application of natural antimicrobials (particularly bacteriocins) to cheese is essential for reducing *L. monocytogenes* contamination.

Nisin, produced by *Lactococcus lactis* ssp. *lactis*, is generally regarded as safe and has been approved as a natural food preservative by the World Health Orga-

nization (Zhu et al., 2015). Nisin has received special attention in the food industry due to its desired inhibitory effect on a large number of gram-positive bacteria, including *L. monocytogenes* and *Staphylococcus aureus*. Nisin can permeabilize membranes through a very efficient, lipid II-dependent, targeted pore-formation mechanism, leading to bacterial death (Breukink and Kruijff, 2006). Previous studies have reported that nisin can be added to various cheeses, such as Cheddar, Camembert, Manchego, Vidiago, and cottage cheeses, to protect against many foodborne pathogens (Aly et al., 2012). However, the negative interaction between bacteriocins such as nisin and food ingredients has been well demonstrated. Binding to food components such as fat and protein can reduce the free bacteriocins in foods (Aasen et al., 2003). As well, the degradation and inactivation of bacteriocins has been attributed to proteolytic activity (Devlieghere et al., 2004). Thus, the direct application of bacteriocins in food can reduce their effectiveness and influence the stability of the food (Malheiros et al., 2012). Encapsulating bacteriocins in nanoliposome systems could circumvent this problem, protecting the bacteriocins and enhancing their stability and bioactivity.

A liposome is an artificial vesicle composed of one or more lipid bilayers incorporating aqueous space. It has been exploited to envelop and release hydrophilic, hydrophobic and amphiphilic bioactive substances due its aqueous and lipid phases (Lin et al., 2015). In our previous studies, a nisin liposome was prepared using a thin film hydration method, but the encapsulation efficiency of the nisin in the liposome system was unsatisfactory. To enhance the encapsulation efficiency, silica, a food additive, has been used as a carrier of bacteriocins because of its high surface area, capacity for encapsulation, and biocompatibility (Bitar et al., 2012). However, aggregation and rupture of the membrane are likely to occur with liposomes, reducing the antibacterial activity of the encapsulated substance (Tan et al., 2013). An edible coating can be used as a carrier for the liposome, so that it is distributed evenly on the edible coating surface.

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Edible coatings obtained from biopolymers are being explored worldwide as a novel food packaging because they are simple, biodegradable, and eco-friendly (Sharma and Rao, 2015). In addition, an edible coating is a semipermeable barrier to gaseous exchange, moisture loss, and oxidative reactions (Pranoto et al., 2005). Among promising candidates, chitosan, a biocompatible polymer, has received widespread attention for its safety, good film-forming ability, excellent mechanical characteristics, and antibacterial performance (Sabaghi et al., 2015). Chitosan coating has been applied in the food industry to maintain food quality.

Based on the above analysis, the incorporation of a nisin-silica liposome into a chitosan edible coating has potential applications in food preservation. However, few studies are available on chitosan-coated nisin-silica liposomes. The main objective of the present study was to investigate the characteristics of nisin-silica liposomes and the sustaining antibacterial activities of chitosan-coated nisin-silica liposomes against *L. monocytogenes* on Cheddar cheese, along with a sensory evaluation.

MATERIALS AND METHODS

Materials and Culture

Cheddar cheese (GRANG'OR Cheddar cheese; protein 25.6%, fat 34.9%, moisture content 37.1%) was purchased from a local supermarket. Nisin (1,000 IU/mg) was obtained from Luoyang Qihong Microbial Technology Co. Ltd. (Luoyang, China). Ammonia solution, absolute ethanol, tetraethylorthosilicate, chitosan, soy lecithin, cholesterol, trichloromethane, polyvinylpyrrolidone, and glycerol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). *Listeria* Chromogenic Medium was purchased from Huankai Microbial Technology Co. Ltd. (Guangzhou, China).

Listeria monocytogenes ATCC 19115, obtained from China General Microbiological Culture Collection Center (Beijing, China), was stored in liquid paraffin wax at 4°C and cultured with shaking at 37°C for 48 h.

Silica Preparation

Silica was synthesized using a modified Stober method (Gangwar et al., 2013). Briefly, 4.0 mL of ammonia solution was mixed in 100 mL of ethanol solution (80%, vol/vol) with a magnetic stirrer for 40 min at 60°C. Then, 4.0 mL of tetraethylorthosilicate was gradually added to the mixture, with continued stirring at 60°C for 90 min. Next, the solution was evaporated in a vacuum oven at 40°C. The silica powder obtained was washed 3 times with double-distilled water to remove

unreacted species. The silica suspension was filtered through 0.1 µm membranes to obtain uniform silica.

Nisin and Nisin-Silica Encapsulation

Nisin liposomes were prepared using a method reported by Malheiros et al. (2010) with minor modifications. Soy lecithin (20 mg/mL) and cholesterol (4 mg/mL) were dissolved in trichloromethane. The organic solvent was evaporated using a rotary evaporator. The resulting thin film was hydrated in PBS (0.03 M, pH 7.2) containing nisin (3, 4, 5, and 6 mg/mL) and polyvinylpyrrolidone (1 mg/mL). Then, the suspension was homogenized by using a cell ultrafine grinding instrument (Ymnl-1000Y; Nanjing Immanuel Instrument Equipment Co. Ltd., Nanjing, China) for 35 min. Finally, the nisin liposome solution was passed through 0.22-µm membranes.

Nisin-silica liposomes were prepared for comparison using the same procedure, except for the addition of nisin-silica instead of nisin. Nisin (3, 4, 5, and 6 mg/mL) and silica (2 mg/mL) were mixed in PBS under stirring for 12 h to obtain a complex of nisin-silica.

Preparation of Chitosan Coating Solutions

To prepare the chitosan stock solution, 1% (wt/wt) chitosan was dispersed in acetic acid solution (1%, vol/vol) under magnetic stirring. Then, the stock solution was diluted with distilled water to obtain 0.1% (vol/vol) chitosan solution. Glycerol (0.5%, vol/vol) was mixed into the chitosan solution (0.1%, vol/vol) as a plasticizer. For chitosan solutions containing liposomes, we added nisin liposomes (20%, vol/vol) or nisin-silica liposomes (20%, vol/vol) under stirring (Jiménez et al., 2014). Finally, all chitosan coating solutions were filtered using a 0.22-µm membrane.

Characterization of Nisin Liposome and Nisin-Silica Liposome

The particle size, polydispersity index (PDI), zeta potential, encapsulation efficiency (EE), and load content (LC) of the nisin and nisin-silica liposomes were investigated. Quartz crystal microbalance was applied to verify that nisin had been encapsulated in the nisin and nisin-silica liposomes. Initially, particle size, PDI, and zeta potential of both liposomes were determined as described in previous studies (Cui et al., 2015, 2016). Then, EE and LC were measured using the bicinchoninic acid assay (Colas et al., 2007). Both liposomes were centrifuged at $9,659 \times g$ for 3 h. The supernatants obtained were added to a France trace protein detec-

tion kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) to measure the concentration (conc.) of unencapsulated nisin. After that, EE and LC were calculated using the following equations:

$$EE (\%) = \frac{\text{overall nisin conc.} - \text{unencapsulated nisin conc.}}{\text{overall nisin conc.}} \text{ and}$$

$$LC (\%wt/wt) = \frac{\text{overall amt. of nisin} - \text{amt. of unencapsulated nisin}}{\text{amt. of lipid}},$$

where amt. = amount. Quartz crystal microbalance was performed using QSX 301 gold sensors on a Q-Sense E4 instrument (Biolin Scientific Co., Ltd., Stockholm, Sweden) to analyze the slight difference in weights between the nisin liposomes (5 mg/mL) and the nisin-silica liposomes (5 mg/mL). For this experiment, 500 μ L samples of each kind of liposome were needed. All experiments were conducted 3 times.

Storage Stability of the Nisin-Silica Liposome

The stability of the nisin-silica liposomes (5 mg/mL) was evaluated during storage. The nisin-silica liposome system was stored at 4°C and 25°C for 60 d. The characteristics of the liposomes were determined over the storage period, including particle size, PDI, zeta potential, EE, and LC.

Anti-listeria Activity of the Nisin-Silica Liposome in a Model Cheese Suspension

Cheese and distilled water (2:8, wt/wt) were blended into a slurry and sterilized. Cheese samples were inoculated with a 1% (vol/vol) of *L. monocytogenes* suspension (approximately 10^5 cfu/mL). Nisin (0.05%), nisin liposomes (20%, vol/vol) or nisin-silica liposomes (20%, vol/vol) were each mixed into separate inoculated samples. Samples containing only *L. monocytogenes* were used as controls. The prepared samples were cultured separately at 25°C and 4°C. Then, the numbers of *L. monocytogenes* were monitored for 7 and 14 d using the plate count method. All experiments were conducted 3 times.

Anti-listeria Activity of the Chitosan-Coated Nisin-Silica Liposome in Model Cheese

The plate colony-counting method was used to evaluate the anti-listeria activity of chitosan-coated nisin-silica liposomes. To prepare model cheese, the cheese suspension (20%, wt/wt) was mixed into PYG agar

medium and sterilized. Then, model cheese was formed using the plate-pouring method and cooled to room temperature. A *L. monocytogenes* suspension (approximately 10^3 cfu/mL) was dispersed well on the surface of the model cheese and maintained for 30 min. Aliquots (500 μ L) of nisin liposome solution (20%, vol/vol), nisin-silica liposome solution (20%, vol/vol), pure chitosan coating solution, chitosan-coated nisin liposome solution, or chitosan-coated nisin-silica liposome solution were coated evenly onto the inoculated model cheese. Inoculated and uncoated model cheese samples were used as controls. Samples were cultured at 25°C and 4°C. Residual bacteria were counted after culturing for 3 d and 5 d.

Anti-listeria Activity of the Chitosan-Coated Nisin-Silica Liposome on Cheese

Fresh cheese samples (30 × 25 × 10 mm) were inoculated with *L. monocytogenes* suspension to obtain a target concentration of approximately 10^3 cfu/g. After 15 min, the inoculated cheese samples were coated with pure chitosan coating solution, chitosan-coated nisin liposome solution, or chitosan-coated nisin-silica liposome solution (Figure 1). Inoculated and uncoated samples were used as controls. All samples were placed in aseptic plastic bags and stored at 25°C for 7 d or 4°C for 15 d. Counts of *L. monocytogenes* were determined using a Listeria Chromogenic Medium. All experiments were conducted 3 times.

Sensory Evaluation

Fresh cheese (30 × 25 × 10 mm) without *L. monocytogenes* was coated with pure chitosan coating, chitosan-coated nisin liposomes, and chitosan-coated nisin-silica liposomes. Uncoated cheese samples were used as controls. Samples were placed in aseptic plastic bags and stored at 4°C for 15 d. The sensory parameters (color, taste, texture, off-flavor, and overall acceptability) of cheese samples were assessed by 25 non-trained panelists (students and researchers) using a hedonic scale of 9 points, where 1 = dislike extremely and 9 = like extremely.

Statistical Analysis

All experiments were conducted 3 times. Results were analyzed using SPSS software (version 22.0; IBM Corp., Armonk, NY). Data are reported as means ± SD. A one-way ANOVA and the Bonferroni statistical test were used to determine significant differences at a significance level of $P < 0.05$.

Figure 1. Release of chitosan-coated nisin-silica liposomes. Color version available online.

RESULTS AND DISCUSSION

Characterization of Nisin Liposome and Nisin-Silica Liposome

We analyzed the primary physicochemical properties of the nisin and nisin-silica liposomes. Particle size and PDI for the 2 kinds of liposome are displayed in Figure 2. Nisin liposomes had a size range of 102.6 nm to 134.3 nm for 3 to 6 mg/mL of nisin. The size of the nisin-silica liposomes ranged from 138.7 nm to 149.2 nm. Compared with the nisin liposomes, incorporation of nisin-silica into the liposome system resulted in a slight increase in average size. The PDI is an index of the width of size distribution, and the PDI for the nisin and nisin-silica liposomes ranged from 0.245 to 0.293 (<0.3), indicating uniformity (Tan et al., 2013). The zeta potential of the 2 types of liposomes in re-distilled water ranged from -37.6 to -44 mV (< -30 mV; Figure 3), indicating that the 2 types of liposome suspensions were stable systems.

The EE and LC of the nisin and nisin-silica liposomes are shown in Figure 4. The EE value of the nisin liposome system ranged from 43.8 to 53.6%, or low. The EE of the nisin-silica liposomes was higher, at 65.5 to 75.7%. The EE of the nisin-silica liposomes reached its highest value at a nisin concentration of 5 mg/mL and declined at 6 mg/mL. Compared with the nisin liposomes, the EE of nisin in the nisin-silica liposomes was sharply increased after it was adsorbed on the silica surface. The LC of the nisin and nisin-silica liposomes ranged from 6.57 to 21.36%, indicating that nisin could be encapsulated into the liposomes within the studied concentrations.

Based on the above analysis, nisin-silica liposomes (5 mg/mL) were selected for further antibacterial testing due to their satisfactory size, EE, and LC.

Quartz crystal microbalance was used to evaluate the slight difference in weights between the nisin liposomes (5 mg/mL) and the nisin-silica liposomes (5 mg/mL). The shift in frequency (ΔF) showed an attractive interaction between the liposome vesicles and the gold sub-

Figure 2. The particle size and polydispersity index (PDI) of nisin liposomes and nisin-silica liposomes with different concentrations of nisin encapsulation. Error bars for values of particle size represent SD = 1.34. Error bars for values of polydispersity index represent SD = 0.0027.

strate (Figure 5). Compared with empty liposomes, the mass of the nisin and nisin-silica liposomes increased markedly, and the nisin-silica liposomes had the largest mass. The results verified that nisin and nisin-silica were encapsulated in the liposome systems.

Storage Stability of Nisin-Silica Liposome

We evaluated the stability of the nisin-silica liposomes (5 mg/mL) stored at 25°C (Table 1) and 4°C (Table 2).

Figure 3. The zeta potential of nisin liposomes and nisin-silica liposomes with different concentrations of nisin encapsulation. Error bars represent SD = 1.28.

Figure 4. The loading capacity and encapsulation efficiency of nisin liposomes and nisin-silica liposomes with different concentrations of nisin encapsulation. Error bars for values of loading capacity represent SD = 0.45. Error bars for values of encapsulation efficiency represent SD = 1.31.

During storage at 25°C, the particle size of the nisin-silica liposomes ranged from 145.3 ± 1.39 nm to 142.8 ± 1.75 nm, but we observed no significant changes over the storage period. The PDI of the liposomes was below 0.3, and the zeta potential was less than -30 mV over time. The EE and LC of the nisin-silica liposomes underwent no significant changes during storage. We observed similar results for the nisin-silica liposomes stored at 4°C. Nisin-silica had practically no leakage

Figure 5. The shift in frequency (ΔF) of empty liposomes, nisin liposomes, and nisin-silica liposomes. Color version available online.

Table 1. Changes in variables (means $\pm$ SD) for nisin-silica liposomes over the storage period at 25°C¹

Storage period (d)	Variable				
	Particle size (nm)	Polydispersity index	Zeta potential (mV)	Encapsulation efficiency (%)	Load content (%)
10	144.9 $\pm$ 1.51	0.269 $\pm$ 0.018	-42.9 $\pm$ 1.23	72.8 $\pm$ 0.83	18.2 $\pm$ 0.61
20	145.3 $\pm$ 1.39	0.248 $\pm$ 0.026	-41.6 $\pm$ 1.89	73.1 $\pm$ 0.55	18.3 $\pm$ 0.58
30	143.6 $\pm$ 1.42	0.257 $\pm$ 0.035	-43.0 $\pm$ 1.57	72.0 $\pm$ 0.67	18.0 $\pm$ 0.86
60	142.8 $\pm$ 1.75	0.231 $\pm$ 0.023	-42.4 $\pm$ 1.61	71.6 $\pm$ 0.78	17.9 $\pm$ 0.77

¹Values within the same column for each variable over the storage period are not significantly different ($P > 0.05$).

Table 2. Changes in variables (means $\pm$ SD) for nisin-silica liposomes over the storage period at 4°C¹

Storage period (d)	Variable				
	Particle size (nm)	Polydispersity index	Zeta potential (mV)	Encapsulation efficiency (%)	Load content (%)
10	146.4 $\pm$ 1.68	0.276 $\pm$ 0.014	-43.1 $\pm$ 1.63	73.5 $\pm$ 0.79	18.4 $\pm$ 0.37
20	145.7 $\pm$ 1.23	0.264 $\pm$ 0.025	-44.9 $\pm$ 2.54	72.8 $\pm$ 0.95	18.2 $\pm$ 0.61
30	143.1 $\pm$ 1.45	0.258 $\pm$ 0.019	-42.7 $\pm$ 2.19	71.9 $\pm$ 0.64	17.9 $\pm$ 0.53
60	144.3 $\pm$ 1.36	0.261 $\pm$ 0.032	-41.8 $\pm$ 3.45	71.1 $\pm$ 0.83	17.8 $\pm$ 0.75

¹Values within the same column for each variable over the storage period are not significantly different ($P > 0.05$).

Table 3. Antibacterial activity (log cfu/g; means $\pm$ SD) of nisin-silica liposomes against *Listeria monocytogenes* in the model cheese suspension stored at 25°C

Treatment	Incubation period (d)							
	0	1	2	3	4	5	6	7
Control	3.62 $\pm$ 0.43	6.36 $\pm$ 0.52 ^c	7.33 $\pm$ 0.69 ^c	7.58 $\pm$ 0.57 ^c	7.83 $\pm$ 0.46 ^c	7.90 $\pm$ 0.55 ^c	8.00 $\pm$ 0.33 ^d	8.04 $\pm$ 0.61 ^c
Nisin	3.58 $\pm$ 0.41	3.32 $\pm$ 0.26 ^a	4.85 $\pm$ 0.34 ^a	6.11 $\pm$ 0.39 ^b	6.83 $\pm$ 0.37 ^b	7.41 $\pm$ 0.52 ^c	7.56 $\pm$ 0.48 ^c	7.65 $\pm$ 0.40 ^c
Nisin liposome	3.61 $\pm$ 0.38	4.39 $\pm$ 0.35 ^b	5.59 $\pm$ 0.27 ^b	6.43 $\pm$ 0.51 ^b	6.76 $\pm$ 0.44 ^b	6.88 $\pm$ 0.58 ^b	6.95 $\pm$ 0.31 ^b	6.99 $\pm$ 0.42 ^b
Nisin-silica liposome	3.59 $\pm$ 0.40	4.08 $\pm$ 0.23 ^b	4.78 $\pm$ 0.41 ^a	5.18 $\pm$ 0.36 ^a	5.34 $\pm$ 0.49 ^a	5.40 $\pm$ 0.57 ^a	5.45 $\pm$ 0.51 ^a	5.48 $\pm$ 0.43 ^a

^{a-d}Distinct letters within the same column indicate significant differences at the same incubation period ($P < 0.05$).

Table 4. Antibacterial activity (log cfu/g; means $\pm$ SD) of nisin-silica liposomes against *Listeria monocytogenes* in the model cheese suspension stored at 4°C

Treatment	Incubation period (d)							
	0	2	4	6	8	10	12	14
Control	3.34 $\pm$ 0.30	3.81 $\pm$ 0.34 ^c	4.51 $\pm$ 0.25 ^b	5.38 $\pm$ 0.39 ^c	6.18 $\pm$ 0.41 ^d	6.54 $\pm$ 0.37 ^d	6.77 $\pm$ 0.48 ^d	6.88 $\pm$ 0.32 ^d
Nisin	3.30 $\pm$ 0.27	2.90 $\pm$ 0.13 ^a	3.99 $\pm$ 0.19 ^a	5.04 $\pm$ 0.27 ^c	5.76 $\pm$ 0.35 ^c	6.10 $\pm$ 0.41 ^c	6.32 $\pm$ 0.34 ^c	6.48 $\pm$ 0.40 ^c
Nisin liposome	3.33 $\pm$ 0.31	3.28 $\pm$ 0.24 ^b	3.83 $\pm$ 0.31 ^a	4.52 $\pm$ 0.25 ^b	4.94 $\pm$ 0.33 ^b	5.41 $\pm$ 0.42 ^b	5.66 $\pm$ 0.28 ^b	5.83 $\pm$ 0.35 ^b
Nisin-silica liposome	3.31 $\pm$ 0.29	3.11 $\pm$ 0.23 ^b	3.69 $\pm$ 0.27 ^a	3.96 $\pm$ 0.34 ^a	4.26 $\pm$ 0.30 ^a	4.36 $\pm$ 0.43 ^a	4.43 $\pm$ 0.34 ^a	4.48 $\pm$ 0.22 ^a

^{a-d}Distinct letters within the same column indicate significant differences at the same incubation period ($P < 0.05$).

Table 5. Antibacterial activity (log cfu/g; means $\pm$ SD) of chitosan-coated nisin-silica liposomes against *Listeria monocytogenes* on cheese stored at 25°C

Treatment	Storage period (d)					
	0	1	2	3	5	7
Control	3.16 ± 0.29	5.78 ± 0.56 ^c	6.47 ± 0.74 ^d	6.97 ± 0.45 ^d	7.34 ± 0.69 ^d	7.69 ± 0.55 ^d
Pure chitosan coating	3.13 ± 0.32	2.62 ± 0.47 ^b	2.31 ± 0.53 ^c	2.14 ± 0.61 ^c	2.17 ± 0.13 ^c	2.05 ± 0.20 ^c
Chitosan-coated nisin liposome	3.14 ± 0.30	2.31 ± 0.33 ^b	1.94 ± 0.28 ^b	1.76 ± 0.36 ^b	1.65 ± 0.18 ^b	1.56 ± 0.14 ^b
Chitosan-coated nisin-silica liposome	3.12 ± 0.27	1.97 ± 0.25 ^a	1.43 ± 0.12 ^a	1.17 ± 0.29 ^a	ND ^{1,a}	ND ^a

^{a-d}Distinct letters within the same column indicate significant differences at the same incubation period ($P < 0.05$).

¹ND = nondetectable; the value is below the limit of detection (1.0 log cfu/g).

Figure 6. The antibacterial activity of chitosan-coated nisin-silica liposomes against *Listeria monocytogenes* in model cheese at 25°C and 4°C. Error bars represent SD = 0.089.

from the liposomes, indicating that the nisin-silica liposomes had good stability during storage.

Anti-listeria Activity of the Nisin-Silica Liposome in a Model Cheese Suspension

We evaluated the antibiotic activity of the nisin-silica liposomes against *L. monocytogenes* in a model cheese suspension at 25°C (Table 3) and 4°C (Table 4). During incubation at 25°C, the numbers of viable bacteria gradually increased. Compared with controls, *L. monocytogenes* in nisin samples increased slowly up to 1 d, but afterward we observed gradual growth of viable bacteria because of nisin deactivation and degradation (Imran et al., 2012). After 3 d, the numbers of *L. monocytogenes* in nisin liposome samples were increased by 2.82 log₁₀ steps, and those in nisin-silica liposome samples were increased by 1.59 log₁₀ steps. We observed similar results for the cheese suspension incubated at 4°C. These findings indicated that the nisin-silica liposomes displayed obviously antibiotic activity against *L. monocytogenes* in a cheese suspension due to its relatively high EE.

Anti-listeria Activity of the Chitosan-Coated Nisin-Silica Liposome in Model Cheese

Figure 6 shows the antibiotic activity of the chitosan-coated nisin-silica liposomes against *L. monocytogenes* in model cheese stored at 25°C and 4°C. Compared with controls (3.25 log cfu/cm²) incubated at 25°C, the numbers of *L. monocytogenes* in the model cheese coated with the nisin liposomes and the nisin-silica liposomes were 2.96 and 2.70 log cfu/cm², respectively. However, the numbers of *L. monocytogenes* on the model cheese coated with pure chitosan coating, chitosan-coated nisin liposomes, and chitosan-coated nisin-silica liposomes were 2.47 log cfu/cm², 2.05 log cfu/cm², and below the limit of detection. We observed similar results for model cheese incubated at 4°C. These findings indicated that chitosan-coated nisin-silica liposomes had better control of *L. monocytogenes* than the other groups.

Anti-listeria Activity of Nisin-Silica Liposome-Embedded Chitosan Coating on Cheese

Results for the antibacterial activity of the chitosan-coated nisin-silica liposomes against *L. monocytogenes* on cheese stored at 25°C and 4°C are shown in Table 5 and Table 6, respectively. Generally, *L. monocytogenes* on cheese grew faster at 25°C than at 4°C. The numbers of *L. monocytogenes* in the controls at 25°C increased rapidly during storage and achieved 7.69 log cfu/g at 7 d. The numbers of *L. monocytogenes* on cheese treated with pure chitosan coating and chitosan-coated nisin liposomes were reduced by 0.96 log cfu/g and 1.49 log cfu/g at 5 d, respectively. However, *L. monocytogenes* growth was distinctly inhibited by the chitosan-coated nisin-silica liposomes at 5 d. Similarly, the numbers of *L. monocytogenes* in the control samples at 4°C increased to 6.65 log cfu/g at 15 d. The growth of *L. monocytogenes* was inhibited by the pure chitosan coating and the chitosan-coated nisin liposomes: numbers at 6 d were reduced by 0.84 log cfu/g and 1.16 log cfu/g, respectively. However, the numbers of *L. monocytogenes* on cheese treated with the chitosan-coated

Table 6. Antibacterial activity (log cfu/g; means ± SD) of chitosan-coated nisin-silica liposomes against *Listeria monocytogenes* on cheese stored at 4°C

Treatment	Storage period (d)					
	0	3	6	9	12	15
Control	3.11 ± 0.28	3.49 ± 0.34 ^c	4.26 ± 0.41 ^d	5.67 ± 0.49 ^d	6.32 ± 0.33 ^c	6.65 ± 0.52 ^c
Pure chitosan coating	3.09 ± 0.31	2.53 ± 0.23 ^b	2.25 ± 0.16 ^c	2.02 ± 0.13 ^c	1.87 ± 0.21 ^b	1.65 ± 0.25 ^b
Chitosan-coated nisin liposome	3.08 ± 0.29	2.27 ± 0.18 ^b	1.92 ± 0.23 ^b	1.69 ± 0.15 ^b	1.57 ± 0.22 ^b	1.49 ± 0.17 ^b
Chitosan-coated nisin-silica liposome	3.06 ± 0.27	1.56 ± 0.14 ^a	ND ^{1,a}	ND ^a	ND ^a	ND ^a

^{a-d}Distinct letters within the same column indicate significant difference at same incubation period ($P < 0.05$).

¹ND = nondetectable; the value is below the limit of detection (1.0 log cfu/g).

Table 7. Sensory evaluation of cheese samples coated with chitosan-coated nisin-silica liposome and before storage (fresh) or stored at 4°C for 15 d

Treatment		Sensory parameter ^{1,2}				
		Color	Taste	Texture	Off-flavor	Overall acceptability
Fresh	Control	4.98 ± 0.34	4.86 ± 0.29	5.08 ± 0.39	5.17 ± 0.48	4.89 ± 0.43
	Pure chitosan coating	4.92 ± 0.41	4.80 ± 0.41	5.06 ± 0.48	5.15 ± 0.56	4.86 ± 0.37
	Chitosan-coated nisin liposome	4.90 ± 0.38	4.82 ± 0.34	5.05 ± 0.37	5.14 ± 0.39	4.90 ± 0.35
	Chitosan-coated nisin-silica liposome	4.93 ± 0.36	4.83 ± 0.32	5.10 ± 0.40	5.18 ± 0.44	4.92 ± 0.42
15 d	Control	4.85 ± 0.36	4.69 ± 0.23	4.87 ± 0.32	4.94 ± 0.37	4.61 ± 0.45
	Pure chitosan coating	4.83 ± 0.31	4.70 ± 0.27	4.90 ± 0.26	4.97 ± 0.41	4.66 ± 0.51
	Chitosan-coated nisin liposome	4.80 ± 0.29	4.72 ± 0.31	4.92 ± 0.30	5.05 ± 0.39	4.70 ± 0.38
	Chitosan-coated nisin-silica liposome	4.82 ± 0.34	4.73 ± 0.28	4.93 ± 0.35	5.08 ± 0.43	4.72 ± 0.34

¹Values within the same column for each variable over the storage period are not significantly different ($P > 0.05$).

²Data are the means (±SD) of a 9-point hedonic scale: 1 = dislike extremely; 2 = dislike very much; 3 = dislike somewhat; 4 = dislike; 5 = neither like nor dislike; 6 = like; 7 = like somewhat; 8 = like very much; 9 = like extremely.

nisin-silica liposomes were below the limit of detection at 6 d, indicating that the chitosan-coated nisin-silica liposomes showed significant antibiotic activity against *L. monocytogenes*. It could be a promising active package for prolonging the shelf stability of cheese.

Sensory Evaluation

Table 7 shows the findings for the sensory evaluation of cheese samples treated with the chitosan-coated nisin-silica liposomes and stored at 4°C. The sensory quality of the samples declined somewhat at the end of storage. However, we observed no significant differences in sensory score among the cheese samples at various storage times. The results indicated that chitosan-coated nisin-silica liposomes could maintain the sensory properties of cheese.

CONCLUSIONS

This study investigated the physicochemical properties of nisin-silica liposomes and the antibiotic activity of chitosan-coated nisin-silica liposomes against *L. monocytogenes* in cheese models, along with a sensory evaluation of the cheese. The results showed that the EE of nisin in nisin-silica liposomes sharply increased after it was adsorbed on the silica surface. Moreover, chitosan-coated nisin-silica liposomes showed the desired antibacterial activity against *L. monocytogenes* without altering the sensory properties of cheese samples. Chitosan-coated nisin-silica liposomes could be a promising active package for food preservation.

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