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Development of an Encapsulation System for the Protection and Controlled Release of Antimicrobial Nisin at Meat Cooking Temperature

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Abstract

Nisin is an antimicrobial peptide produced by *Lactococcus lactis* spp. *lactis* widely investigated for use in foods as a natural antimicrobial. However, its effective use in meat products is restricted notably by its reaction with meat constituents (including glutathione) in raw meat. The purpose of this study was to develop an encapsulation system that would optimize nisin activity when used in meat. To achieve this goal, an encapsulation in dipalmitoylphosphatidylcholine (DPPC) liposomes was developed. DPPC liposomes were formed in phosphate buffer with or without nisin. The encapsulation efficiency of nisin in liposomes was greater than 46 ± 2%. The median size of nisin-loaded liposomes was 495 nm, compared to 170 nm for empty liposomes. The liposomes containing nisin were stable for up to 7 days at 4°C but a zone of inhibition was observed afterwards. Stability of the liposome to heat was also tested and demonstrated that above 37°C nisin was released from the melted liposomes to form zones of inhibition. Activity of free and encapsulated nisin was tested in raw and cooked ground beef (71°C). Free nisin lost its activity in raw beef but DPPC-encapsulated nisin remained active and was released upon melting of the liposome during heat treatment.

Keywords: antimicrobial, delivery system, encapsulation, liposome, meat, nisin

1. Introduction

Bacteriocins are antimicrobial peptides, which are produced by bacteria to inhibit the growth of other closely related organisms and can be potentially used as natural preservative (Cleveland, Montville, Nes, & Chikindas, 2001; de Arauz, Jozala, Mazzola, & Penna, 2009). Nisin is a 3.5 kDa cationic peptide produced by *Lactococcuslactis* subsp. lactis (O'Sullivan, Ross, & Hill, 2002). It is used as a food preservative because of its antimicrobial activity against several pathogenic Gram-positive bacteria that can be present in food, such as *Listeria monocytogenes*, *Staphylococcus aureus*, spores of *Bacilluscereus* and *Clostridium botulinum* (Najjar, Chikindas, & Montville, 2007). However, nisin is not active against Gram-negative bacteria, yeast or molds (Delves-Broughton, Blackburn, Evans, & Hugenholtz, 1996) unless a chelating agent is used concurrently (Cutter & Siragusa, 1995). Nisin antibacterial activity targets the cytoplasmic membrane where it inhibits peptidoglycan synthesis and supports the formation of pores (Bonev, Chan, Bycroft, Roberts, & Watts, 2000; Wiedemann et al., 2001). Nisin is a permitted food additive in more than 50 countries, including the US and Europe, where it is used notably in processed cheeses, dairy products and canned foods (Delves-Broughton et al., 1996).

Despite its extended uses, addition of nisin to meat products has been largely unsuccessful, unless used at very high concentrations (Stevens, Sheldon, Klapes, & Klaenhammer, 1991) or post-treatment (Davies et al., 1999; Rose, Sporns, Stiles, & McMullen, 1999). Several reports attribute the inactivation of nisin in meat as being mainly due to reactionwith meat components (Chung, Dickson, & Crouse, 1989), sensitivity to food enzymes (Shiba, Krushna, & Supratim, 2011), poor solubility at the pH of meat (Scannell, Hill, Buckley, & Arendt, 1997),

high bacterial loads (Scott & Taylor, 1981) and interaction with phospholipids (Henning, Metz, & Hammes, 1986). These difficulties are primarily linked to the hydrophobic nature of nisin and its poor solubility at neutral pH (Henning et al., 1986; Scott & Tylor, 1981; Stringer, Dodd, Morgan, & Waites, 1995; Delves-Broughton et al., 1996). At room temperature, nisin A is relatively stable in the pH range of 2 to 6, with highest stability at pH 3 (Rollema, Kuipers, Both, De Vos, & Siezen, 1995). A sharp decrease in stability is observed at pH 7 and 8. The pH of fresh meat is in the range of 5.5 to 5.8 depending on the animal species. Furthermore, dark, firm and dry (DFD) meats, and some processed meats, like bologna, have pH values above 6 (Faucitano et al., 2010; Viuda-Martos, Ruiz-Navajas, Fernándes-López, & Pérez-Álvarez, 2010). So not all added nisin is expected to be solubilized in meat matrices.

Nisin is inactivated by the presence of proteases, titanium dioxide, sodium metabisulfite (Delves-Broughton, 2005) or by enzymatic reaction with glutathione, a low molecular mass (307 Da) thiol compound found in meat tissues (Rose et al., 1999; Rose, Palcic, Sporns, & McMullen, 2002). In contrast, when nisin is applied to cooked meat, there is no inactivation. The concentration of glutathione in beef, chicken and pork is generally high with 156 to 627 nmol/g wet weight compared to raw fish (21 nmol/g wet weight; Jones et al., 1992). Under optimal reaction temperatures three glutathione molecules can bind to one nisin molecule and multiple dehydro-residues are involved, resulting in the loss of antimicrobial activity (Rose et al., 2002). Tilokavichai, Jindaprasert, Pilasombut, Sethakuland Swetwiwathana (2011) showed that nisin A and nisin Z is inactivated by an enzymatic reaction with 250 mM glutathione at a temperature of approximately 30 to 32°C. On the contrary, the same authors showed that glutathione does not affect the activity of pediocin PA-1 and plantaricin W at 30 to 32°C and 4°C (Tilokavichai, Jindaprasert, Pilasombut, Sethakul, & Swetwiwathana, 2012).

In this study, we investigated the possibility of protecting nisin by encapsulation in liposomes to provide a temperature controlled release system that enhances the efficacy and stability of nisin added to raw meat (here raw beef), similar to what is achieved during the direct acidification of meat (e.g., pepperoni; Barbut, 2005). Encapsulation in liposomes allows for the timely liberation of acids as the liposomes melt during cooking. This approach has already been successfully applied to protect nisin in a cheese matrix (Benech, Kheadr, Laridi, Lacroix, & Fliss, 2003) and other food systems (Malheiros, Micheletto, da Silveira, & Brandelli, 2010). Liposomes are spherical bi-layer vesicles formed by the dispersion of polar lipids in aqueous solvents and may consist of single or multiple bilayers composed of polar lipids (Mertins, Sebben, Pohlmann, & da Silveira, 2005). The most commonly used phospholipid for liposome formation is lecithine but its transition phase occurs at 25°C (Mertins, Sebben, Schneider, Pohlmann, & da Silveira, 2008). However, other phospholipids, like dipalmitoylphosphatidylcholine (DPPC) with a transition phase at 42°C, are more convenient for meat cooking applications. Before nisin can be used to its full potential in various meat systems, it is important to develop strategies that can effectively protect it from inactivation.

2. Materials and Methods

2.1 Bacterial Cultures and Growth Conditions

Several bacterial strains were tested as indicator organism of nisin activity (Table 1). The two strains of *Listeria monocytogenes* were kindly provided by Health Canada and were originally isolated from meat. The strains of *L. innocua* and *Pediococcus acidilactici* UL5 were obtained from the Department of Food Science and Nutrition and the strain of *Clostridium sporogenes* ATCC19404 from the Department of Biochemistry and Microbiology at Université Laval. Stock cultures were stored at -80°C in Lactobacilli de Man, Rogosa and Sharpe broth (MRS; BD Difco, Franklin Lakes, New Jersey, USA; pH 6.7 ± 0.2) for *P. acidilactici* UL5, in Brain Heart Infusion (BHI; BD Difco; pH 7.2 ± 0.2) for *Listeria* spp. and in BHI supplemented with 5 g/l yeast extract, 0.1% L-cysteine (BHIS; pH 6.7 ± 0.2) for *C. sporogenes* ATCC19404. All frozen cultures were supplemented with 20% glycerol (FisherBiotech, Fairlawn, NJ, USA) as a cryoprotectant. Prior to experimental use, working cultures were individually thawed and subcultured (1% (v/v)) daily in their respective broth media described above for a minimum of two and a maximum of seven consecutive days. Cultures were incubated overnight at 30°C for *Listeria* spp. and *P. acidilactici* UL5, and for 4 days at 37°C in anaerobic condition for *C. sporogenes* ATCC19404 (Forma Anaerobic System Model Covered 1025 S/N 13930-475, Thermo Scientific, Inc., Marietta, OH, USA).

Table 1. Antimicrobial activity of nisin against different strains

Strains	Nisinactivity[b]		
	AU/ml	Diameter (mm)[c]	MIC (μg/ml)[d]
C. sporogenes ATCC19404	800	13.1 ± 0.9	10.4 (5.2 - 10.4)
L. innocua HPB13	400	14.9 ± 2.0	5.2
L. monocytogenes HPB2371	400	13.7 ± 0.5	2.6 (2.6 - 5.2)
L. monocytogenes HPB2569	400	10.4 ± 1.2	2.6 (2.6 - 5.2)
P. acidilactici UL5	3200	28.8 ± 3.3	0.7

[a] The concentration of pure nisin in the solution used to produce the liposome is 83.3 μg/ml.

[b] All experiments were repeated three times.

[c] Zone of inhibition produced by the undiluted nisin solution used to produce the liposome (83.3 μg/ml).

[d] MIC was determined as the lowest concentration required for complete growth inhibition of the target. microorganism and are expressed as the median, the range of values are in parentheses. In the first well, after proper dilution with cells and media, nisin concentration was 41.7 μg/ml.

2.2 Antimicrobial Activity

Nisin activity was determined by the agar diffusion methods as previously described by Gratia (1946, cited by Dajani, & Wannamaker, 1976; Rose et al., 1999). Minimum inhibitory concentrations (MIC) were determined using the microplate assay previously described by Mota-Meira, LaPointe, Lacroix andLavoie (2000). For the agar diffusion method, 15 ml of the appropriate soft agar (0.75% (w/v) agar) was melted, inoculated at 1% (v/v) with a fully grown culture of the indicator organism, mixed and poured onto a pre-solidified agar plate (1.5%). A 20 μl aliquot of the antimicrobial solution to be tested was spotted at different concentration on the inoculated soft agar and was dried in a biosafety cabinet (highest concentration of nisin tested was 83.3 μg/ml). Activity was expressed as arbitrary activity units (Ahn & Stiles, 1990a, b); the dilution factor was multiplied by 50 to bring the arbitrary activity unit to 1 ml. Zones of inhibition were measured using a caliper (Mitutoyo Corporation., Ltd., Aurora, IL, USA) after 18 h of incubation for *Pediococcus* and *Listeria* and after 24 h to 48 h for *Clostridium*, under the same growth conditions as described above. For the microplate dilution method, 125 μl of each medium was added to each well of a sterile in 96-well U-bottom plate (Dynex Technologies LTD., Guernsey, Channel Islands, UK). The same volume (125 μl) of the nisin solution (83.3μg/ml) to be tested was added to the first well and serial dilutions (1:2) were made. The optical density (OD_{600}) of the overnight cultures was adjusted to 0.1 with fresh broth (Benchmark, Bio-Rad, Hercules, CA, USA) and corresponds to a 0.5 McFarland standard (1-2 x 10^8 CFU/ml). The microplates were incubated for 18 h for *Pediococcus* and *Listeria* and after 24 h to 48 h for *Clostridium*. Absorbance was read (OD_{600}) with a spectrophotometer (Thermo-Spectronic, UV-1 model, Thermo Electron Corporation, USA). The MIC was determined as the lowest concentration required for complete growth inhibition of the target microorganism determined by comparing OD_{600} value with the negative control without cells. Since *P. acidilactici* UL5 was the most sensitive strains amongst those evaluated, it was selected to follow nisin activity (Table 1).

2.3 Liposome Preparation and Encapsulation Efficiency

Liposomes were prepared from DPPC (Avanti Polar Lipids, Alabaster, AL, USA) using the method of Taylor, Gaysinsky, Davidson, Bruce and Weiss (2007) with the following modifications. The lipids were first dispersed in chloroform and dried under N_2 to form a lipid film on the wall of glass reaction tubes. Samples were desiccated overnight under vacuum to remove solvents. For nisin-loaded liposomes, Nisaplin (2.5% (w/w) in NaCl and denatured milk solids) was kindly provided by DuPont (formerly Danisco, New Centery, KS, USA) and dissolved in 20 mM HCl (pH 2) to obtain an acidic stock solution of pure nisin at a concentration of 250 μg/ml. Throughout this manuscript, all levels of nisin are reported as pure nisin. The nisin stock solution was immersed in boiling water for 5 min, filter sterilized through a 0.22 μm surfactant-free cellulose acetate filter (SFCA, 28 mm syringe filter; Corning Inc., Corning, NY, USA). Lipid films were rehydrated with nisin diluted in 0.1% phosphate buffered saline (PBS; 0.017 M KH_2PO_4, 0.05 M Na_2HPO_4, and 1.5 M NaCl at pH 7.4; Biowhittaker, Rockland, ME, USA) to a final nisin and lipid concentration of 83.3 μg/ml and 10 mM, respectively. After rehydration, liposomes were frozen in liquid nitrogen for 10 s, then gently thawed in water (25°C) for 10 s, and immediately immersed in a 50°C water bath for 15 s. This thermal cycle was repeated four

times to favor encapsulation. Liposomes were then held at 50°C for 20 min to ensure that phospholipids were above their gel-liquid crystalline phase transition temperature (T_m) before sonication to promote uniform sizes and to reduce the size of the vesicles. Multilamellar vesicles (MLV) were exposed to five cycles of sonication (Sonic Dismembrator, Model 500, Fisher Scientific, Pittsburgh, PA, USA) for 1 min, followed by 3 min of cooling on ice (Malheiros et al., 2010). The sample remained on ice for 15 min and then the liposomes were separated from unencapsulatednisin by ultracentrifugation (model L8-70 M ultracentrifuge; Beckman, Palo Alto, CA, USA) at 85 000 x g for 1 h at 20°C, washed twice, and recentrifuged (Benech et al., 2002). The pelleted liposomes were dissolved in 0.1% PBS at pH 7.4 and the supernatants were retained to determine the encapsulation efficiency by protein concentration analysis. To determine the activity of encapsulated nisin, the pellet was heated above the transition temperature of the lipid (60°C) to release nisin and the preparation was centrifuged to keep only the released nisin in the supernatant. Total protein was determined using the BCA Protein Assay Kit-Reducing Agent Compatible (Thermo Scientific, Rockford, IL, USA).

2.4 Stability of Liposomes Stored at 4°C

The liposomes were stored at 4°C and their stability was followed for 7 days by measuring the zone of inhibition produced on a lawn of *P. acidilactici* as described above. The size distribution of empty and nisin-loaded DPPC liposomes was analyzed by photon correlation spectroscopy using a Nicomp Submicron particle sizer apparatus (Model 370, HIAC/ROYCO Instruments, Menlo Park, CA, USA) equipped with a 25 mW helium/neon laser. Different temperatures (4, 25, 37, 42, 50, 63 and 71°C) were tested for the liberation of nisin. Liposomes were heated for 30 min. Nisin liberation was detected by the inactivation of the indicator organism as described above.

2.5 Activity of Free and Encapsulated Nisin Incorporated in Raw and Cooked Beef

To determine the activity of nisin in extra lean ground beef (≤ 10% fat; pH 5.61 ± 0.03) free (control) and encapsulated nisin were added to the meat samples (83.3 µg/g or 3200 AU/ml). The meat was hand massaged to evenly distribute the added nisin in the meat matrix. Raw beef containing free or encapsulated nisin was tested immediately after mixing or after storage at 4°C for various lengths of time (15, 30 and 60 min, and 24h) to allow the reaction of nisin with raw beef constituents. Residual nisin activity in the raw beef was evaluated by inhibition of the indicator strains as described below. Residual nisin activity was also evaluated after the raw beef was cooked to a core temperature of 71°C (no holding time). For cooking, samples of raw beef (7 g) containing free or encapsulated nisin were placed in glass tubes 1 cm in diameter and were heated in a high precision (± 0.001°C) circulating programmable water bath (Cole-Palmer Polystat Heated Circulating Bath, Cole-Parmer Canada Inc., Anjou, QC, Canada) set at 80°C. Empty liposome without nisin was also evaluated as a negative control. The temperature of the meat was followed using a data logger equipped with a type T thermocouple (Food tracker MultiPaq21, Datapaq Inc., Wilmington, MA, USA). After treatment, samples were cooled in an iced water bath (4°C). To detect residual nisin activity when added to raw beef, before or after cooking, 50 ml of MRS agar (1.5% (w/v) agar) was poured into a 100 ml beaker. A sterile glass tube was used to punch a hole in the center of the solidified agar to create a well of 1.5 cm in diameter and 4 cm deep. The raw or cooked meat sample was placed in the well and covered with 8 ml of soft MRS agar (0.75% (w/v) agar) inoculated with the indicator organism.The beaker was then incubated for 24 h at 30 ± 1°C. The size of the inhibition zone obtained was measured with a caliper.

3. Results and Discussion

3.1 Liposome Encapsulation Efficiency

As determined by protein dosage, the encapsulation efficiency of nisin in DPPC liposomes was greater than 46 ± 2% and higher than the efficiency previously determined for commercial proliposome H (34.6%), as reported by Laridi et al. (2003). Previous studies demonstrated that encapsulation efficiency was greater in phosphatidylcholine (PC) prepared liposomes than in phosphatidylglycerolprepared liposomes (Laridi et al., 2003; Were, Bruce, Davidson, & Weiss, 2004). Images obtained by light microscopy before and after sonication (Figures 1A and B, respectively) indicated that liposomes varied greatly in size; after sonication, liposomes became smaller. Encapsulation of nisin resulted in an increase in liposome size, confirming its insertion in the liposome. The median size of nisin-loaded liposomes was 495 nm, compared to 170 nm for empty liposomes formed in PBS buffer 0.1%, pH 7.4 without nisin. Taylor et al. (2007) showed that encapsulation of pure nisin in phosphatidylcholine liposomes resulted in vesicles with an approximate size of 310 nm, compared to 103 nm for empty liposomes.

3.2 Liposome Stability

Liposomes containing nisin were stored at 4°C and the release of nisin was monitored over time to evaluate the liposome stability. Nisin remained within the liposome for up to 7 days at 4°C as demonstrated by the absence of an inhibition zone when spotted on a lawn of the indicator organism (Figure 1C). After 7 days at 4°C, nisin began to be released from the liposome in its active form as indicated by the zone of inhibition observed (Figure 1D). Nisin acts as a cationic detergent leading to the formation of pores in the lipid bilayers of membranes (Harris, Daeschel, Stiles, & Klaenhammer, 1989) and this characteristic suggests that nisin may be able to form pores in the liposome bilayer similar to those observed in the cytoplasmic membranes of sensitive organisms. Because nisin is released in its active from the liposome after a certain time, it may be advantageous for use in a food system for controlled release during storage. Malheiros et al. (2010) have demonstrated that the decrease in antimicrobial activity of nisin containing liposomes is time dependent and up to 25% of the activity can remain inside the liposome after 10 days at 4°C.

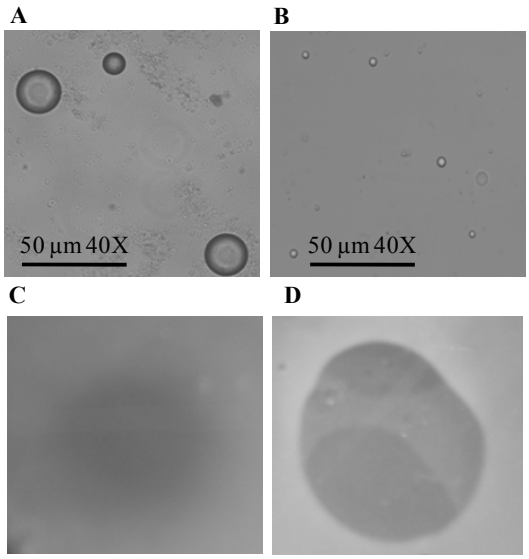

Figure 1. Optical microscopy images and stability of liposomes stored at 4°C

Images include liposomes (A) before and (B) after sonication, respectively. After sonication, liposomes became smaller. No clear zone of inhibition was observed from liposome containing nisin up to 7 days of storage at 4°C suggesting that if nisin was released, it was not enough to inhibit the indicator strains, *P. acidilactici,* (C) but after 7 days, zones of inhibition were observed suggesting that sufficient quantity of nisin began to be released from the liposome in its active form to inhibit the indicator strains, *P. acidilactici,* (D). Photographs C and D were taken against a black background.

3.3 Temperature Stability of Liposomes

The controlled release of nisin from DPPC liposomes was tested at different temperatures (4, 25, 37, 42, 50, 63 and 71°C). Liposomes (500 µl) were heated for 30 min and 20 µl was spotted on inoculated soft agar plates (Figure 2). At 4 and 25°C, nisin was completely retained as no inhibition zone was observed (Figures 2A and B). When the temperature was increased to 37°C and above, nisin was released, resulting in zones of growth inhibition (Figure 2C-F). These results show that temperatures ≥ 37°C alter the surface properties of the liposomes to allow nisin to be effectively released.

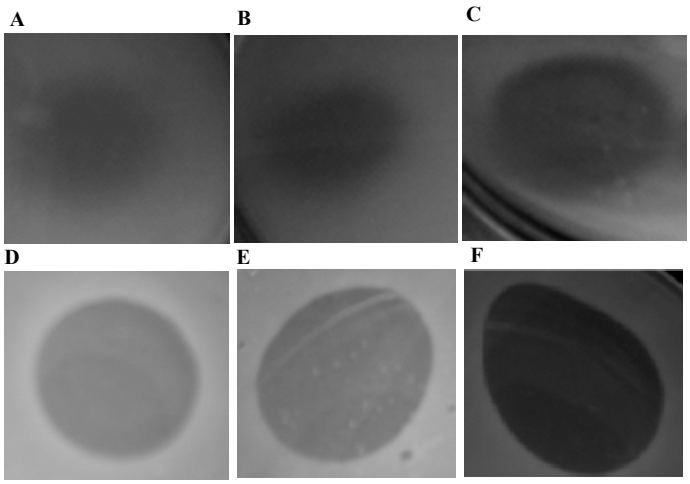

Figure 2. Liposome stability after heat treatments at different temperatures

The release of nisin from liposomes was tested at 4 (A), 37 (B), 42 (C), 50 (D), 63 (E) and 71°C (F). Liposomes were heated and then the solution spotted on the inoculated soft agar plates. At 4 and 25°C, no clear inhibition zone was observed suggesting that if nisin was released, it was not in sufficient quantity to inhibit the indicator strains, *P. acidilactici,* (data not shown for 25°C). When the temperature was ≥ 37°C, nisin was released, resulting in zones of growth inhibition. Experiments were repeated three times. Photographs were taken against a black background.

3.4 Protection of Nisin Activity in a Beef Model

Empty liposome as well as free and encapsulated nisin were mixed into ground beef and stored at 4°C for various lengths of time before heating to a core temperature of 71°C. Nisin activity was detected as a growth inhibition of the indicator organism. For a free nisin concentration of 83.3 μg/g (3200 AU/g), no inhibition zone was observed with raw or cooked beef after 30 to 60 min of contact with the ground beef (Figures 3A and B). No zone of inhibition was observed with the empty liposome either before or after heat treatment (data not shown) indicating that HCl at the concentration used in our experiments had no contribution to the inhibitory effect observed. A minimum time of contact was required before the free nisin added to raw beef was completely inactivated. As indicated above, previous reports indicated that the loss of free nisin activity in meat could be due, notably, to the composition of the meat, such as the presence of proteases (Delves-Broughton, 2005), and the reaction of nisin with meat constituents such as glutathione (Rose et al., 1999). Encapsulated nisin incubated with raw beef did not produce inhibition zones of the indicator strains, no matter the length of exposure (Figure 3C). After cooking to a core temperature of 71°C, sufficient quantity of nisin was released in cooked beef to form inhibition zones of the indicator strains (Figure 3D). The size of inhibition zones varied from 20 to 25 mm (zone edge to opposite zone edge). Similar results as Figure 3 line C and D were observed even after 24 h of incubation before and after cooking (data not shown). These results suggest that nisin was protected inside the liposomes and that the cooking temperature releases it upon liposome melting as was observed in the temperature stability experiment (see section 3.3). The observed activity after cooking supports the results of Rose et al. (1999) who found that nisin remained active when added to cooked meat. DPPC encapsulated nisin retained its activity in cooked beef. The temperature increase during cooking likely led to the timely denaturation of glutathione and other meat constituents involved in nisin inactivation (Freeman, Huntley, Meredith, Senisterra, & Lepock, 1997) concurrent with the release of nisin from the melted liposome (fusion temperature of DPPC is 63°C). Degnan and Luchansky (1992) monitored the activity of free pediocinAcH in slurries of beef tallow and beef muscle (25% in dH_2O). Significant loss of pediocin AcH activity was observed, but more activity was retained in heated slurries (100°C, 3 min) compared to unheated slurries. They suggested that enzymes degrading pediocin were inactivated by the heat treatment or that reaction substrates were denatured and no longer able to react with pediocin.

Concerns regarding the high levels of nitrite in cured meat have resulted in the research for alternatives. Although, unlike nitrite, nisin has no functionality in meat with respect to color, antioxidant properties, flavor etc., its antimicrobial activity, especially towards sporeformers, may allow the reduction of the nitrite/nitrate concentration currently used in meat systems since most of it is added to fulfil microbial preservation (Saucier,

1999). Its production by a dairy starter culture, *L. lactis* spp. *lactis,* is attractive since it could meet consumer demand for more natural additives for use in processed foods. However, the inactivation of nisin in raw meat systems is well documented and has been a major setback, delaying its wider application as an antimicrobial for use in meat systems. In this study, we developed the production of liposomes in hydrated film to encapsulate nisin and to protect it against inactivation prior to cooking, therefore, allowing it to retain its activity in cooked meat although initially added to raw beef. Figure 4 is a schematic of how the encapsulation system works. Free nisin in meat becomes inactive through reaction with meat constituents, including glutathione, as previously reported (Rose et al., 1999; Rose et al., 2002). By liposome encapsulation, nisin is protected inside the liposome and is released with increasing temperature. Increasing temperature destabilizes the liposome membrane and likely denatures meat constituents, including glutathione, which are then no longer able to react with nisin, leaving nisin in its active form in the meat matrix.

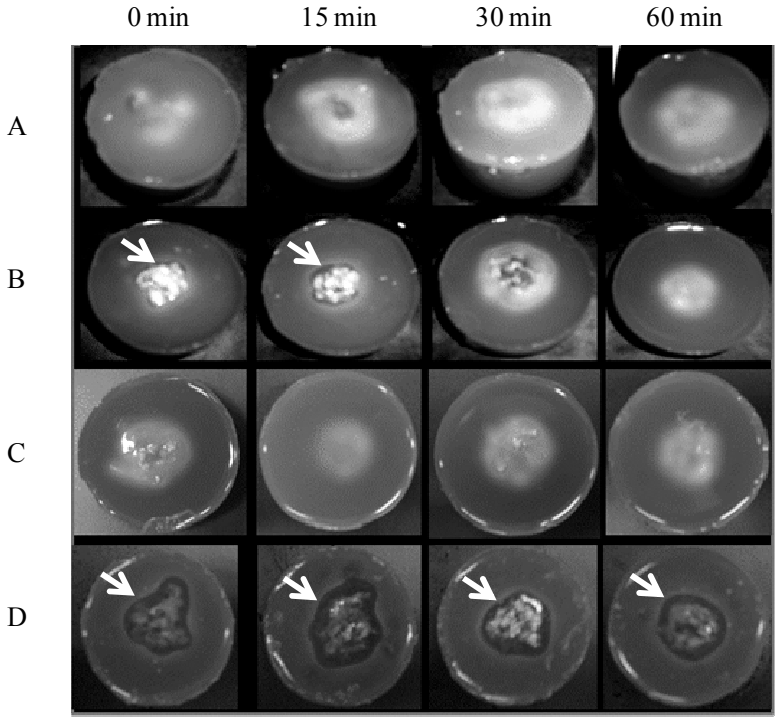

Figure 3. Activity of free (A, B) and DPPC encapsulated nisin (C, D) in ground beef

Nisin was tested at 83.3 µg/g (3200 AU/ml) of meat. Free and encapsulated nisin were tested in raw beef immediately after mixing or after 15, 30 and 60 min of contact prior (A, C) or after heat treatment to a core temperature of 71°C (B, D). A minimum time of contact (30 min and more) was required before the free nisin added to raw beef was completely inactivated (B). Encapsulated nisin incubated with raw beef did not produce inhibition zones no matter the length of exposure (C) indicating that nisin remained encapsuled until the heat treatment was applied and the liposome melted (D). Arrows indicate zones of inhibition. The experiments were repeated three times.

The results obtained in this study suggest that other sensitive molecules could be protected from heat inactivation by encapsulation in liposomes provided that their chemical characteristics are compatible with the liposome constituents. Now that we have demonstrated that this strategy works in beef, further studies are required to improve level of encapsulation, to develop procedures to produce liposomes without the use of solvent (here chloroform), and to validate the efficacy of encapsulated nisin to control spoilage and pathogenic organisms in various meat systems.

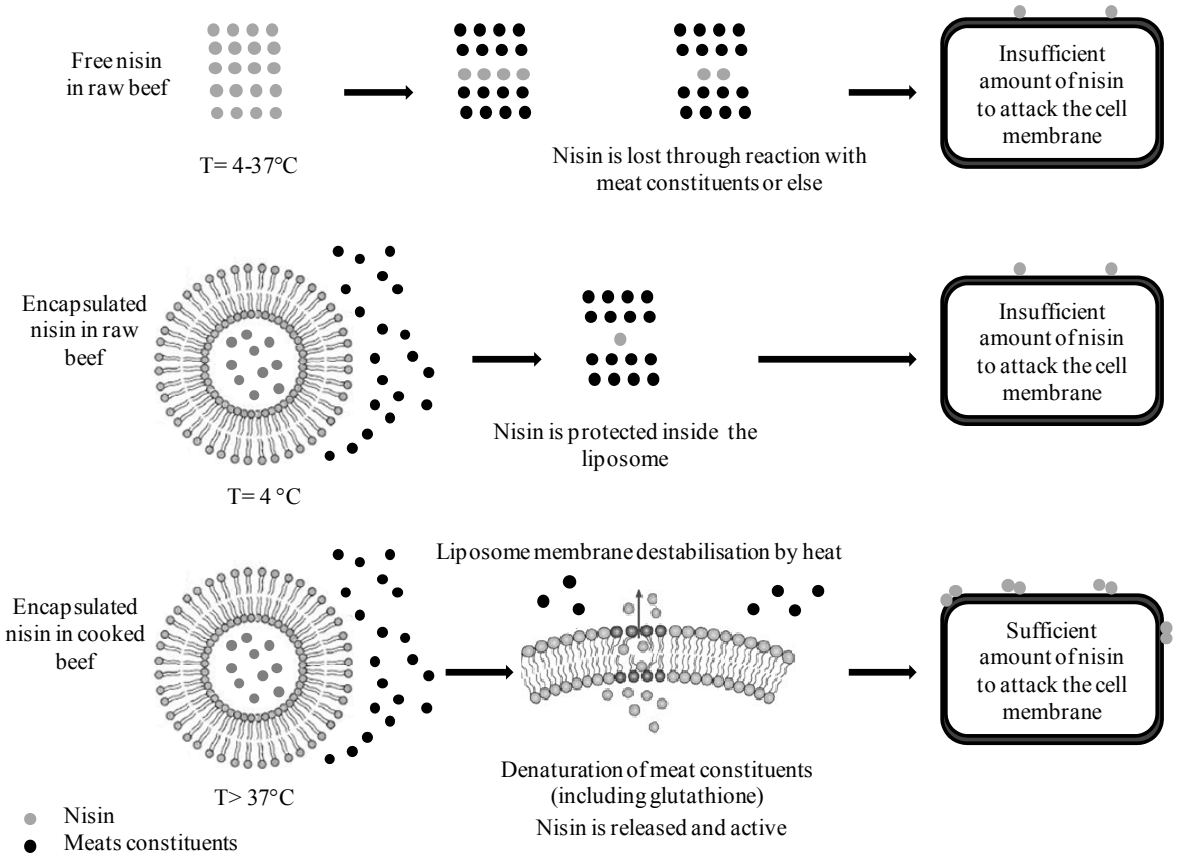

Figure 4. Schematic of nisinprotection mechanism by encapsulation in liposomes

Free nisin in raw beef is inactivated, resulting in an insufficient amount of nisin to attack the cell membranes of target microorganisms. Nisin is protected inside the liposome until cooking is applied. Upon heat treatment, nisin is released from the liposome in its active form so the target organisms can be inactivated.

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