Protection and Controlled Release of Nisin in Meat during Cooking using Liposome Encapsulation

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SUMMARY

The effective use of the antimicrobial peptide nisin in meat products is restricted, notably, by its reaction with meat constituents including glutathione and proteases in raw meat. The purpose of this study was to prepare liposomes for meat/food applications in order to maximize the activity of nisin during food processing and storage. To achieve this goal, an encapsulation procedure in dipalmitoylphosphatidylcholine (DPPC) liposomes was developed. Nisin was protected and released in its active form upon cooking when encapsulated in liposomes prepared from DPPC while free nisin, not protected in liposomes, lost its activity. During cooking, inactivation of glutathione, notably, occurs at a lower temperature than the melting temperature of the DPPC liposomes allowing nisin to be released and to retain its activity in the meat.

WHY DOES NISIN NEED TO BE PROTECTED IN RAW MEAT?

Nisin is a 3.5 kDa cationic peptide produced by Lactococcus lactis subsp. lactis (O'Sullivan et al. 2002). It is used as a food preservative because of its antimicrobial activity against several pathogenic bacteria that can be present in foods, such as Listeria monocytogenes, Staphylococcus aureus, spores of Bacillus cereus and Clostridium botulinum (Najjar et al. 2007). It is widely studied and accepted for use in foods in many countries but not in Canada yet. Because nisin is a peptide, it is inactivated by the presence of proteases in raw meat (Delves-Broughton, 2005). Furthermore, Prof. L.M. McMullen's team from the University of Alberta demonstrated that nisin is inactivated by enzymatic reaction with glutathione, a low molecular mass (307 Da) thiol compound found in meat tissues (Rose et al. 1999; Rose et al. 2002). In contrast, the U of A team showed that when nisin is applied to cooked meat, there is no inactivation. 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).

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, similar to what is achieved during the direct acidification of meat (e.g., pepperoni). The most commonly used phospholipid for liposome formation is lecithine but its transition phase occurs at 25°C, which is rather low (Mertins et al. 2008). However, other phospholipids, like dipalmitoylphosphatidylcholine (DPPC) with a transition phase at 42°C, are more convenient for protecting bioactive molecules in meat cooking applications. Indeed, DPPC transition temperature (42°C) is higher than the temperature necessary to inactivate the enzymatic reaction of nisin with glutathione (30-32°C; Tilikavichai et al. 2011). 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

EXPERIMENTAL PROCEDURES

The standard methods for liposomes preparation generally involve the use of solvents for the solubilisation of phospholipids (e.g., chloroform; Taylor et al. 2007) but later on we were able to effectively encapsulate nisin without the use of such solvent and allow the liberation of active nisin in

meat. The food grade liposomes were prepared by hydrating phospholipids directly into PBS buffer (0.017 M KH2PO4, Na2HPO4 0.05 M and 1.5 M NaCl at pH 6.4), HCl (20 mM, pH 2) or other organic acids (e.g. lactic) by vortexing, hence avoiding the formation of hydrated film with chloroform. Nisin, in the form of Nisaplin (2.5% (w/w) in NaCl and denatured milk solids), was kindly provided by DuPont (formerly Danisco, New Centery, KS, USA). It was dissolved in acidic conditions (HCl or organic acid pH 2-3) to obtain a stock solution of 250 µg/mL and then further diluted at a concentration of 83.25 µg/mL of nisin or higher. Liposomes were purified by centrifugation (85,000 x g, for 1 h at 25°C) and resuspended in appropriate buffer.

To determine the efficacy of nisin in extra lean beef (≤ 10% fat), encapsulated nisin was added to the meat samples and hand massaged to evenly distribute the encapsulated nisin in the meat matrix. Raw meat was stored at 4°C for various times (0, 15, 30, 60 min and 24 h) until it was tested directly in raw meat or cooked to a core temperature of 71°C. Briefly, samples of raw meat (7 g) containing the encapsulated nisin were placed in a glass tubes of 1 cm in diameter and 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) at 80°C. 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 determine the release of nisin, 50 mL of MRS agar (0.75% agar) was poured into a small 100 mL beaker. Once the agar was solidified, a hole was punctured in the middle using a sterile glass tube to make a well of 1.5 cm in diameter and 4 cm in height. The raw or cooked meat sample was placed in the well and covered with 8 mL of soft MRS agar (0.75% agar) inoculated with the indicator organism (1%). The beaker was incubated for 24 h at 30 ± 1□C. The size of the inhibition zone obtained was measured with a caliper (Mitutoyo Corporation., Ltd., Aurora, IL, USA).

OUTCOMES AND PERSPECTIVES

Nisin activity was detected as a growth inhibition of the indicator organism. Liposomes containing nisin and prepared under various conditions (e.g., pH, buffers, with or without solubilisation of phospholipids in chloroform, etc.) were able to protect and release active nisin after cooking. Here as an example (Figure 1), 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 (Figure 1A and B). A minimum time of contact was required before the free nisin added to raw beef was completely inactivated. This explains why nisin has been largely unsuccessful in meat products unless used at very high concentrations. Encapsulated nisin incubated with raw beef did not produce inhibition zones of the indicator strains, no matter the length of exposure (Figure 1C). However, 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 1D). The size of inhibition zones varied from 20 to 25 mm (zone edge to opposite zone edge). Similar results as Figure 1 line C and D were observed even after 24 h of incubation before and after cooking. Hence, nisin is protected inside the liposomes and released upon cooking. The observed activity after cooking support the results of Rose et al. (1999) who demonstrated that nisin remained active when added to cooked meat. Indeed, the increasing temperature during cooking most probably led to the timely denaturation of glutathione and other meat constituents (Tilikavichai et al. 2011) with the release of nisin from the melted liposome (fusion temperature of DPPC is 63°C; Figure 2). Here, nisin was encapsulated but other sensitive molecules could be protected from heat inactivation by encapsulation in liposomes provided that their chemical characteristics are compatible with the liposome constituents.

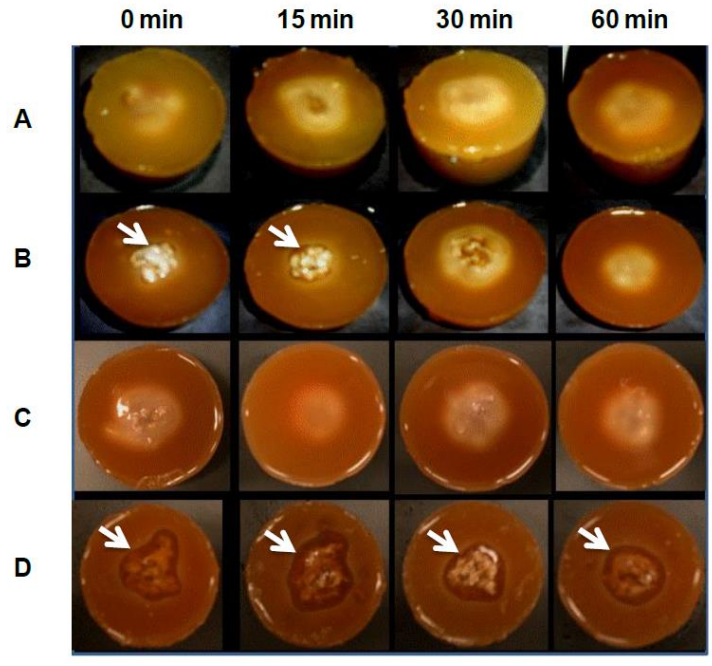

Figure 1. Activity of free (A, B) and DPPC encapsulated nisin (C, D) in ground beef. Nisin was tested at 83.3 µg/g (3200 AU/ml) of meat. Free and encapsulated nisin were tested in raw beef immediately after mixing or after 15, 30 and 60 min of contact prior (A, C) or after heat treatment to a core temperature 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. Pediococcus acidilactici UL5 was used as the indicator organisms to determine nisin activity. The experiments were repeated three times.

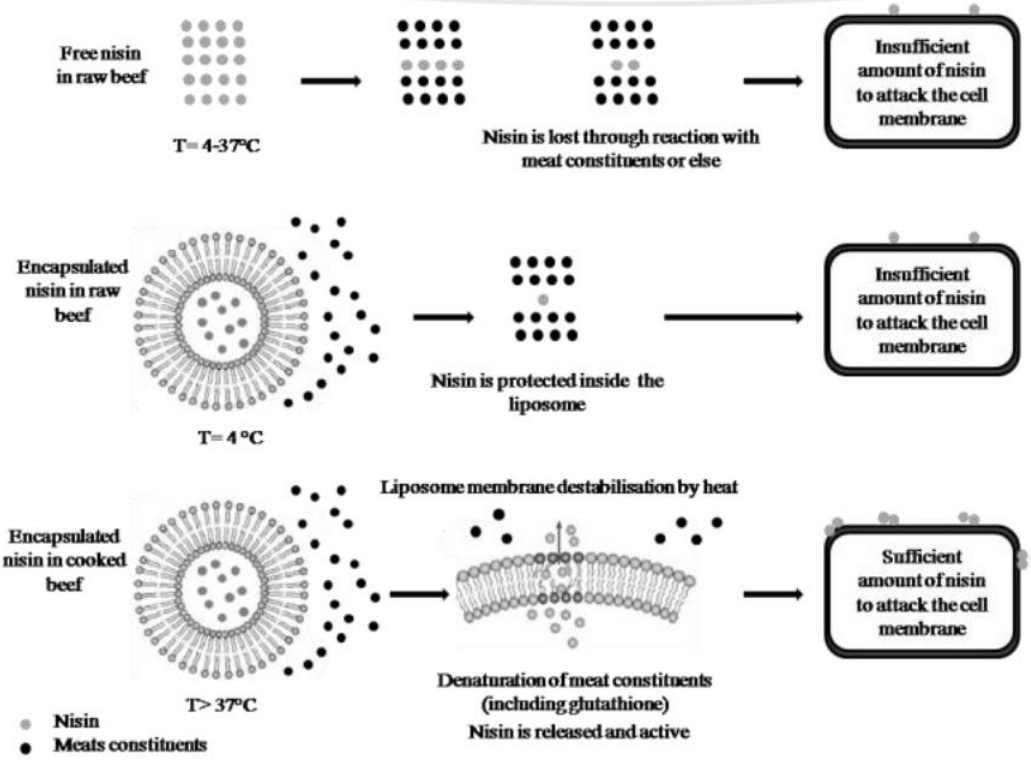

Figure 2. Schematic of nisin protection mechanism by encapsulation in liposomes. Free nisin in raw beef is inactivated, resulting in an insufficient amount of nisin to attack the cell membranes of target microorganisms. Nisin is protected inside the liposome until cooking is applied. Upon heat treatment, nisin is released from the liposome in its active form so the target organisms can be inactivated.

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