ANTIMICROBIAL EDIBLE MIRCOENCAPSULATED COATING CONTAINING NISIN AGAINST *LISTERIA MONOCYTOGENES* IN COOKED HAM

Tanzina Huq[1], Dominic Dussault[1], Stephane Salmieri[1], Canh Le Tien[1], Bernard Riedl[2], Jean Bouchard[3] and Monique Lacroix[1]

[1]Research Laboratories in Sciences Applied to Food, Canadian Irradiation Centre (CIC), INRS-Institut Armand-Frappier, University of Quebec, 531 Boulevard des Prairies, Laval, Québec, H7V 1B7, Canada. [2]Département des sciences du bois et de la forêt, Faculté de foresterie, géographie et géomatique, Université Laval, Québec, G1V0A6, Canada.

[3]FPInnovations, 570 Boulevard St. Jean, Pointe-Claire, Québec, H9R 3J9, Canada

Abstract –The present study was undertaken to develop an effective edible antimicrobial coating containing microencapsulated nisin to control the growth of *Listeria monocytogenes* in cooked ham slices. Nisin (0.125, 0.25 and 0.5% w/w) was microencapsulated into alginate–NCC (nanocrystalline cellulose) microbeads. The cooked ham slices were coated with the microbeads, inoculated with *L. monocytogenes* (~3 log CFU/g) and stored at 4°C under vacuum packaging for 28 days. The microencapsulated nisin beads showed better antimicrobial efficacy than the non-microencapsulated beads. The alginate-NCC microbead coating with 0.125, 0.25 and 0.5% of nisin significantly reduced (3.65, 4.51 and 6.56 log CFU/g, respectively) the growth of *L. monocytogenes* on cooked ham slices after 28 days of storage at 4°C compared to the control (coating without antimicrobial). This study demonstrates the effectiveness of using a coating with microencapsulated nisin beads to control the growth of *L. monocytogenes.*

Key Words – Nisin, microbead gel coating, *Listeria monocytogenes.*

I. INTRODUCTION

Contamination of ready-to-eat (RTE) meat with *L. monocytogenes* occurs mainly at post processing, and consumption of these products without further heating is common. Although RTE meats contain salts such as sodium chloride, nitrite and nitrate that have antimicrobial activities, they do not inhibit the growth of *L. monocytogenes* during storage at refrigerated temperatures [1]. *L. monocytogenes*, a Gram-positive, non-spore forming rod shaped bacterium, has long been established as an important foodborne pathogen but it is now considered a pathogen of major concern because of the severity of the illness [2].

Nisin is an antimicrobial peptide or bacteriocin, produced by several strains of *Lactococcus lactis* and recognized as GRAS by the United States Food and Drug Administration (FDA) as stated in the Code of Federal Regulations (CFR section 184.1538). Nisin has been used throughout the world to preserve meat [3]. This antimicrobial protein exhibits inhibitory activity against spore-forming bacteria and other Gram-positive spoilage and pathogenic bacteria including *L. monocytogenes* [4].

Sodium alginate is the salt of alginic acid, a linear (1–4) linked polyuronic acid extracted from brown sea weed. Alginate has been used as a carrier of nisin to coat the poultry meat [5] and beef [6]. Edible coatings can act as effective carriers of antimicrobials for treating food surfaces which represent the typical point of entry of pathogens and likely location of maximum microbial contamination [7].

The main objective of this study was to observe the antimicrobial activity of microencapsulated nisin at different concentrations against *L. monocytogenes* in cooked ham during storage at 4°C.

II. MATERIALS AND METHODS

Microencapsulation of Nisin

An aqueous suspension containing 2% (w/v) alginate [guluronic acid (Eq) or glucuronic (Ax) content ~ 65 - 70%; mannuronic acid content ~5 - 35%, Sigma-Aldrich Canada Ltd., Oakville, ON, Canada] and 5% (w/w) NCC (NCC was supplied from FPInnovations, Pointe-Claire, Quebe, Canada)

were homogenized using a Ultra-Turrax TP18/1059 homogenizer (Janke & Kunkel, Staufen, Germany) at 23°C and 25000 rpm for 1 min. A 0.5% w/v Lecithin (American Lecithin Company, ALC, USA) was slowly added to the alginate suspension and heated to 60°C for 30 min. The pH of alginate-lipid suspension was adjusted to 7.5 to 8.0 by using 0.1M NaOH. The pH of this suspension was lowered to 5.5-6.0 by using 0.05M lactic acid. A 0.125, 0.25 and 0.5% w/w of Nisin (2.5%, Profood, IL, USA) was mixed in 0.01M $CaCl_2$ solution. Then the antimicrobial gel beads were obtained by addition of 0.01M $CaCl_2$ containing nisin to the suspension under vigorous stirring at room temperature [8].

Bacteria Culture

Listeria monocytogenes HPB five strains (2569, 2558, 2371, 2812 and 1043) were used in this experiment. The microorganisms were kept frozen at -80°C in tryptic soy broth (TSB, Difco, Becton Dickinson, MD, USA) containing glycerol (10% v/v). Before use, the stock cultures were resuscitated through 2 consecutive 24 h growth in TSB at 37°C to obtain the working cultures containing approximately 10^9 CFU/mL.

Preparation of Ham Samples

Ground lean pork meat was purchased from a local grocery store (Metro, Laval, Quebec, Canada). Ground ham was cooked with different preservatives such as sodium chloride, triphosphate, erythorbate and nitrite salt (BSA Food Ingredients, St-Leonard, Quebec, Canada) for about 1 h at 162.7°C in cooking oven. Following cooking the ham was removed from the oven and placed at 4°C for 24 h. Then the cooked ham was sliced and 5 ml of microencapsulated nisin on each side of the ham slice was added. The coated cooked ham slice was then inoculated with ~3 log CFU/g *L. monocytogenes* and vacuum packaged within 24 h of production. The samples were stored at 4°C up to 28 days.

Bacterial Enumeration

Each ham sample was homogenized for 2 min in sterile peptone water (0.1%, wt/vol; Difco, Becton Dickinson) in a Lab-blender 400 stomacher (Seward Medical, London, UK). From this homogenate, appropriate serial dilutions were prepared in 0.1% peptone, and 100 µl of each dilution was spread plated on PALCAM (Alpha Biosciences, MD, USA), which were incubated for 48 h at 37°C. Bacterial counts were expressed as log CFU/g of ham.

Statistical Analysis

All bacterial counts were log10 transformed prior to statistical analysis. Three samples per day of analysis were used for each group. An analysis of variance (ANOVA) and multiple comparison tests of Duncan's were used to compare all the results. Differences between means were considered significant when the confidence interval was smaller than 5% ($P \leq 0.05$). The analysis was performed by the PASW statistics 18 software (SPSS Inc., Chicago, IL, USA).

III. RESULTS AND DISCUSSION

The antimicrobial effect of nisin microencapsulation is represented in Figure 1. The effectiveness of nisin to prevent the growth of *L. monocytogenes* was dependent on the nisin concentration. *L. monocytogenes* present on the control increased significantly ($P \leq 0.05$) by a maximum of 8.25 log CFU/g over a period of 4 weeks of storage at 4°C. The microencapsulated nisin showed a better effect compared to both the non-microencapsulated and control coating. Coating with 0.125, 0.25 and 0.5% (w/w) microencapsulated nisin reduced the counts by 2.59, 1.49 and 3.09 log CFU/g, respectively compared to the non microencapsulated coating. The growth of *L. monocytogenes* was lower than the detection limit (100 CFU/g) for the microencapsulated beads containing 0.5% w/w nisin.

The external surface of cooked ham slice acts as a primary site of contamination by *L. monocytogenes* during processing (slicing and packaging) and the equipment, personnel, and other surfaces with which the product comes in contact, become contaminated and in turn these serve as secondary sources of contamination. *L. monocytogenes* contamination of preserved products such as cooked ham renders it a high-risk product as pronounced uninhibited growth of the organism is a potential risk. *L. monocytogenes* is a psychrotrophic pathogen that is able to grow on cooked ham at refrigeration temperature of 4°C [9].

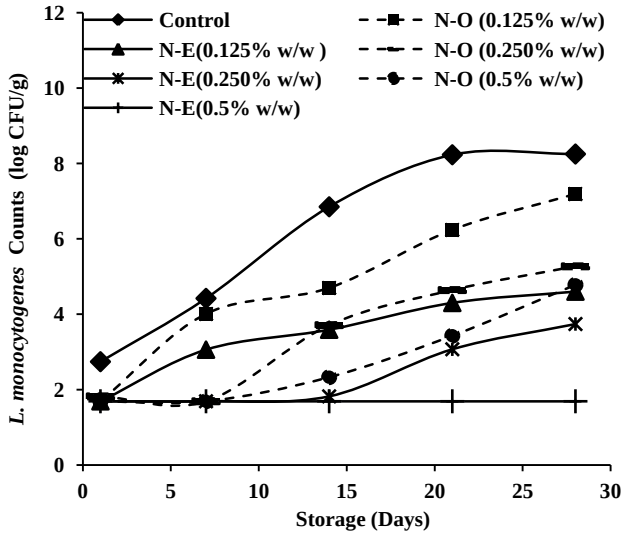

Figure 1. Counts of *L. monocytogenes* on vacuum packaged cooked ham slices coated with nisin microencapsulated at 4°C
(N-O represents nisin without microencapsulated and N-E represents microencapsulated nisin)

It is surmised that preservatives and antimicrobials directly applied on the surface may diffuse much faster throughout the product lowering the local surface concentration to sub-active levels; edible antimicrobial coatings could maintain the necessary preservative concentration at the product surface for a relatively longer period of time [7].

The microencapsulated nisin significantly ($P \leq 0.05$) inhibit *L. monocytogenes* compared to the non-microencapsulated. It was reported that the inhibitory effect of the growth of *Staphylococcus aureus* by nisin-containing modified alginate films and beads on beef. The author suggested that the hydrophobic and biodegradable films or beads incorporating various amounts of nisin could be used to control the growth of pathogens or microorganisms responsible for spoilage at the surface of ground beef or other meat products [10]. Zein coatings incorporating nisin, sodium lactate and sodium diacetate alone or in combination were also shown to be effective inhibitors of *L. monocytogenes* on full-fat turkey frankfurters during a 28-day storage period at 4°C [11].

IV. CONCLUSION

In conclusion, the results obtained in this study showed that microencapsulated nisin coatings can improve the safety of sliced cooked ham by inhibiting the growth of *L. monocytogenes*. Our findings further demonstrated the importance of microencapsulation of antimicrobial agents compared to the conventional direct addition method.

ACKNOWLEDGEMENTS

This research was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) and by FPInnovations (Pointe-Claire, Quebec, Canada) through the RDC program. The authors would also like to thank BSA Food Ingredients s.e.c./l.p. for providing salt ingredients (Montreal, Quebec, Canada). Tanzina Huq is the recipient of a scholarship from Fondation Armand-Frappier.

REFERENCES

1. Mbandi, E. & Shelef, L.A. (2002). Enhanced antimicrobial effects of combination of lactate and diacetate on *Listeria monocytogenes* and *Salmonella* spp. in beef bologna. International Journal of Food Microbiology 76: 191– 198.

2. Neetoo, H., Ye, M. & Chen, H. (2010). Bioactive alginate coatings to control *Listeria monocytogenes* on cold-smoked salmon slices and fillets. International Journal of Food Microbiology 136: 326–331.

3. Cutter, C. N., & Siragusa, G. R. (1997). Growth of *Brochothrix thermosphacta* in ground beef following treatments with nisin in calcium alginate gels. Food Microbiology 14: 425–430.

4. Schillinger, U., Chung, H.S., Keppler, K. & Holzapfel, W.H. (1998). Use of bacteriocinogenic lactic acid bacteria to inhibit spontaneous nisin-resistant mutants of *Listeria monocytogenes* Scott A. Journal of Applied Microbiology 85: 657–663.

5. Natrajan, N. & Sheldon, B. W. (2000). Inhibition of *Salmonella* on poultry skin using protein- and polysaccharide-based films containing nisin formulation. Journal of Food Protection 63: 1268–1272.

6. Cutter, C. N. & Siragusa, G. R. (1996). Reduction of *Brochothix thermosphacta* on beef surfaces following immobilization in calcium gels. Letters in Applied Microbiology 23: 9–12.

7. Coma, V., (2008). Bioactive packaging technologies for extended shelf life of meat-based products. Meat Science 78: 90–103.

8. Rajaonarivony, M., Vauthier, C., Couarraze, G., Puisieuxa, F. & Couvreur, P. (1993). Development of a New Drug Carrier Made from Alginate. Journal of Pharmaceutical Sciences 82: 912-917.

9. Stekelenburg, F.K. & Kant-Muermans, M.L.T. (2001). Effects of sodium lactate and other additives in a cooked ham product on sensory quality and development of a strain of *Lactobacillus curvatus* and *Listeria monocytogenes.* International Journal of Food Microbiology 66: 197–203.

10. Millette, M., Tien, C.L., Smoragiewicz, W. & Lacroix, M. (2007). Inhibition of *Staphylococcus aureus* on beef by nisin-containing modified films and beads. Food Control 18: 878–884.

11. Lungu, B. & Johnson, M.G. (2005). Fate of *Listeria monocytogenes* inoculated onto the surface of model turkey frankfurter pieces treated with zein coatings containing nisin, sodium diacetate, and sodium lactate at 4°C. Journal of Food Protection 68: 855–859.