MEAT PROCESSING

ENHANCING THE SHELF-LIFE AND SAFETY OF CURED MEATS.

Richard Holley and Alexander Gill, University of Manitoba

Background:
The refrigerated shelf-life of meats has been considerably extended through the use of modern packaging technology. Delicatessen meats routinely have a minimum shelf-life of about 49 days, fresh beef 45 days, and fresh pork 42 days, provided proper refrigeration, sanitation, and packaging requirements are met. Much longer shelf-lives are possible for refrigerated meats where stringent levels of sanitation and refrigeration are used at the packing plant in conjunction with more expensive packaging techniques and lower refrigeration temperatures during the distribution and retail display.

In spite of the advances made in extending the shelf-life of these products, there are periodic reports of large outbreaks of food borne illness developing through consumption of these delicatessen products. These incidents plus the occasional premature spoilage of the meats are of concern because the cooking step used during processing is normally sufficient to kill pathogens and most spoilage bacteria. However, these organisms may gain entry to products after cooking and during packaging.

Since there is both regulatory and consumer reluctance to accept products laced with preservatives, we have been working, through this project to develop new natural antimicrobial mixtures and improve the methods by which they are used to prevent bacterial growth in cured meat products. By applying antimicrobials at the meat surfaces - either as a separate coating or attached to the packaging film- in order to inhibit bacteria where they cause problems in these products, we expect to be able to reduce the concentrations of inhibitors required to achieve shelf-life and safety goals.

Objectives:
To demonstrate that application of lysozyme: nisin, 1:3 plus EDTA (500 $mg.kg^{-1}$) at the cured meat surface will improve antimicrobial activity and extend product shelf-life.

To broaden the antimicrobial action of lysozyme against Gram negative bacteria by the use of natural spice extractives and by formation of conjugates with other natural antimicrobials.

Results and Discussion:

In this project we have been evaluating the use of a mixture of lysozyme and nisin in a ratio of 1:3 with a chelator, ethylenediamine tetraacetic acid (EDTA), to inhibit several spoilage and pathogenic bacteria in commercially prepared ham and bologna.

After laboratory screening tests we formulated the inhibitor complex directly into raw meat batters at a concentration of 500 $mg.kg^{-1}$, cooked the meat in sausage casings, which was then inoculated with one of; *Brochothrix thermosphacta*, *Escherichia coli* 0157:H7, *Lactobacillus curvatus, Lactobacillus sakei, Leuconostoc mesenteroides*, *Listeria monocytogenes, Salmonella typhimurium, Serratia grimesii* or *Shewanella putrefaciens*. The growth of the bacterial population was monitored during vacuum packaged storage of the products at 8°C.

Antimicrobial treatment reduced initial populations of *B. thermosphacta* and *Lc. mesenteroides* on both meats. Treatment of ham and bologna prevented growth of *B. thermosphacta*, to week 4. Treatment reduced growth of *Lb. curvatus* on ham and bologna, to week 3. Treat-

ment of bologna reduced the growth of *Lc. mesenteroides* and *L. monocytogenes* for 2 weeks. Treatment of ham reduced growth of *E. coli* O157:H7 for 4 weeks. On treated ham the growth of *S. typhimurium* was increased from week 3. No difference was observed between control and treatment samples with the other organisms.

To improve potency, we then applied the inhibitor at the surface of the sausages as either an aqueous solution or gelatin gel, inoculated with bacteria, vacuum packaged, and again stored the ham and bologna at 8°C.

Treatment with an aqueous antimicrobial solutions of antimicrobials was observed to be consistently less effective than coating with an antimicrobial gel and was rejected as a delivery method.

To evaluate the effectiveness of an antimicrobial gel treatment, cooked ham and bologna sausage was prepared and received one of three treatments: no coating (control); coating with a 7% (w/v) gelatin gel (gel-control), or coating with a 7% gelatin gel containing 25.5 g/liter lysozyme:nisin, 1:3, plus 25.5 g/liter EDTA (gel-treated); which is equivalent to 447 mg/kg^{-1} of antimicrobial per total mass for a 10g sample coated with 0.2g of gelatin gel.

The samples were then inoculated with one of six test organisms; *B. thermosphacta, E. coli* O157:H7, *Lb. sakei, Lc. mesenteroides, L. monocytogenes,* or *S. typhimurium*. Inoculated samples were vacuum packed and stored at 8 °C for 4 weeks.

The antimicrobial gel treatment had an immediate bactericidal effect up to 4 log CFU/cm^2 on the four Gram positive organisms tested (*B. thermosphacta*, *Lb. sakei, Lc. mesenteroides* and *L. monocytogenes*) and inhibited the growth of these organisms over the 4 weeks of storage. The antimicrobial gel treatment also had a bactericidal effect on the growth of *S. typhimurium* over the period of storage. The numbers of *E. coli* O157:H7 on ham were reduced by 2 log CFU/cm^2 following treatment with both antimicrobial-containing and non-antimicrobial gels over the 4 week storage period, but no effect was observed upon the growth of *E. coli* O157:H7 on bologna.

To improve the spectrum of lysozyme activity against the full range of test bacteria, experiments were conducted where lysozyme was conjugated with two types of fatty acids (lauric or palmitic) or a spice derivative, cinnamaldehyde. These reactions led to a change in the solubility of the lysozyme molecule permitting it to dissolve better in the lipid-like phase of the bacterial cell wall/membrane complex and so theoretically improve its antimicrobial activity. The "conjugates" of lysozyme were chemically prepared and when tested had some activity against *Brochothrix thermosphacta,* but their activity against many of the test organisms was not as great as the lysozyme-nisin mixture in meats. These conjugate tests were conducted at 24°C rather than at refrigerator temperatures (as preliminary tests) and if they had been conducted at 4°C might have yielded improved results. Since inhibitory action was not as great as had been predicted and because use of modified lysozyme would require costly toxicity testing before permission for their use in food could be given, these tests were not pursued further.

Results from this project indicate that use of lysozyme-nisin in a surface treatment of vacuum packaged cooked cured meats can significantly improve product shelf-life and safety.

Conclusion:

Surface treatment of cured meats with lysozyme-nisin-EDTA antimicrobial mixture at packaging was a most effective way to delay growth and even killed some spoilage and pathogenic bacteria when these organisms were added before packaging and stored at 8°C. Surface application was significantly more effec-

tive than when the inhibitor was mixed as an ingredient before cooking. Both the potency and spectrum of inhibitor activity were increased by surface application. Inhibitory action against *Listeria monocytogenes* was most notable and activity was quite effective. Since this human pathogen was responsible for the largest meat recall to date in North America (Sara Lee Corporation, Jan. 1999, 35 million pounds of cured meat) both the inhibitor and method of its application may find application to prevent future economic loss and reduce morbidity and mortality. Since the inhibitor could have had improved activity against *E. coli*, particularly on bologna, future work to broaden its spectrum of activity could prove valuable.

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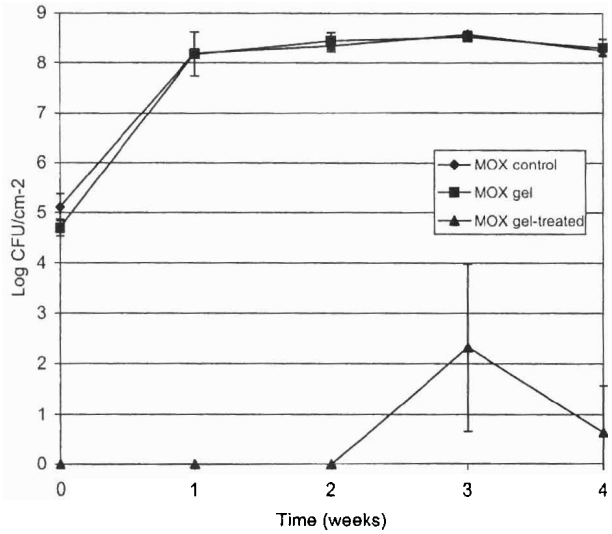

Figure 1. Growth of *Listeria monocytogenes* on ham without treatment (control) or dipped in 7% (w/v) gelatin gel with lysozyme:nisin, 1:3 (25.5 $g.L^{-1}$) plus EDTA (25.5 $g.L^{-1}$) (gel-treated) or without inhibitors (gel). All samples were fully cooked before inoculation with the test organism, vacuum packed and stored at 8 °C. Organisms were recovered on modified oxford agar (MOX). Vertical bars represent one standard deviation interval. Data points without visible vertical bars have a standard deviation of <0.1 Log CFU.cm

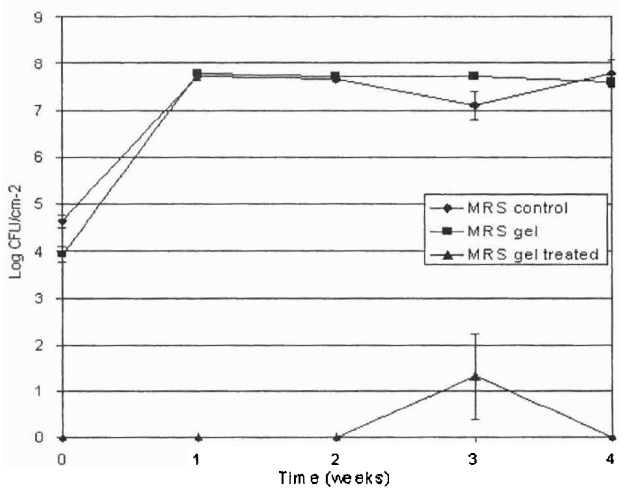

Figure 2. Growth of *Leuconostoc mesenteroides* on ham without treatment (control) or dipped in 7% (w/v) gelatin gel with lysozyme:nisin, 1:3 (25.5 $g.L^{-1}$) plus EDTA (25.5 $g.L^{-1}$) (gel-treated) or without inhibitors (gel). All samples were fully cooked before inoculation with the test organism, vacuum packed and stored at 8 °C. Organisms were recovered on de Man Rogosa Sharpe agar (MRS). Vertical bars represent one standard deviation interval. Data points without visible vertical bars have a standard deviation of <0.1 Log CFU.cm