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Inhibition of bacterial growth on ham and bologna by lysozyme, nisin and EDTA

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Abstract

Ham and bologna sausages were prepared with or without addition of 500 mg kg^{-1} lysozyme:nisin, 1:3, and 500 mg. kg^{-1} EDTA. Sausages were inoculated with one of; *Brochothrix thermosphacta*, *Escherichia coli* O157:H7, *Lactobacillus sakei*, *Lactobacillus curvatus*, *Leuconostoc mesenteroides*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Serratia grimesii* or *Shewanella putrefaciens*, vacuum packed and stored for 4 weeks at 8°C. Plate counts were made on selective and nonselective media. Inhibitor 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. Treatment of bologna reduced 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 other organisms. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Lysozyme; Nisin; EDTA; Antimicrobial; Cured meat spoilage; Bacterial pathogen

1. Introduction

Lysozyme is a 14.6 kDa single peptide protein (Proctor & Cunningham, 1988), which possesses enzymatic activity against the β(1–4) glycosidic linkages between *N*-acetylmuramic acid and *N*-acetylglucosamine found in peptidoglycan. Peptidoglycan is the major component of the cell wall of both Gram positive and Gram negative bacteria. Hydrolysis of the cell wall by lysozyme can damage the structural integrity of the cell wall and result in the lysis of bacterial cells. Lysozyme is of interest for use in food systems as it is a naturally occurring enzyme that is produced by many animals, including man, and has activity against a cellular structure specific to bacteria (Proctor & Cunningham).

The bacteriocin, nisin, has been used as an antimicrobial in foods since the 1960's (for a review see Delves-Broughton, Blackburn, Evans & Hugenholtz, 1996). The activity of nisin, like lysozyme, is specific for bacterial cells. Nisin is produced by the lactic acid bacterium (LAB) *Lactococcus lactis*. Nisin is most effective as an antimicrobial against LAB and other Gram positive organisms, notably clostridia species (Delves-Broughton et al.). The mechanism of nisin activity has been shown to involve alteration of the cell membrane of sensitive organisms resulting in leakage of low molecular weight cytoplasmic components and destruction of the proton motive force (PMF) (Bruno, Kaiser & Montville, 1992). Two models for nisin activity have been postulated. The "detergent-disruption" model is one in which the nisin molecule is thought to disrupt the physical structure of the cell membrane in a manner similar to detergents. An alternative theory is the "poration complex" model which postulates the formation of an oligomeric pore in the cell membrane by the aggregation of nisin monomers to yield cytoplasmic leakage (Montville, Winkowski & Ludescher, 1995).

It has been recognized since the 1960's that susceptibility of Gram negative organisms to lysis by lysozyme can be increased by the use of membrane disrupting agents, such as detergents and chelators (Shively and Hartsell, 1964a,b). Ethylenediaminetetraacetate (EDTA), a chelator, can have an antimicrobial effect by limiting the availability of cations and can act to destabilize the

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cell membranes of bacteria by complexing divalent cations which act as salt bridges between membrane macromolecules, such as lipopolysaccharrides (Shelef & Seiter, 1993; Varaa, 1992).

Previous experiments in all purpose tween and brain heart infusion broths have indicated that a combination treatment of lysozyme, nisin and EDTA may be effective in controlling the growth of organisms of concern in the spoilage and safety of cured meat products (Gill & Holley, 1998).

In this series of experiments, commercially formulated ham or bologna batter was used to prepare sausages with 500 mg kg^{-1} lysozyme:nisin, 1:3 plus 500 mg kg^{-1} EDTA (treatment), or without any inhibitors (control). The products were cooked, cut into 14 mm thick coins and then inoculated with one of 9 organisms: *Brochothrix (B.) thermosphacta*, *Escherichia (E.) coli* O157:H7, *Lactobacillus (Lb.) sakei*, *Lactobacillus (Lb.) curvatus*, *Leuconostoc (Lc.) mesenteroides*, *Listeria (L.) monocytogenes, Salmonella (S.) typhimurium*, *Serratia (Ser.) grimesii,* or *Shewanella (Sh.) putrefaciens*. The products were then vacuum packed and incubated at 8°C. The microbial populations on the meats were monitored by plating onto selective and non-selective media, to determine whether there was a difference between the population levels on control and treated samples.

Studies have been published by a number of authors on the use of lysozyme or nisin as antimicrobials in a wide variety of food products (for reviews see, Delves-Broughton, et al., 1996; Proctor & Cunningham, 1988). Studies on the use of lysozyme or nisin as antimicrobials for use in meat products have been primarily concerned with surface treatments applied to fresh meat products (Cutter & Siragusa, 1995, 1997; Murray & Richard 1997; Sheffet, Sheldon & Klaenhammer, 1995). Application of antimicrobial treatments to cured meat products is an area that has received little attention. However, cured meat products may be an excellent system in which to use lysozyme, nisin and EDTA combination treatments, since the presence of other growth restrictive chemicals and conditions, such as nitrite and NaCl (Gill & Holley, 1998) may increase the effectiveness of antimicrobial treatment against spoilage flora and pathogens.

2. Materials and methods

2.1. Cultures used, incubation conditions and selective media

B. thermosphacta B2 was from Dr. G. Greer, Agriculture and Agrifood Canada (AAFC) Research Station, Lacombe, AB (brain heart infusion broth, BHI, aerobic). *E. coli* O157:H7 E318 was a human clinical isolate provided by Dr. R Johnson, Health Canada, Guelph, ON, resistant to 30 ppm nalidixic acid (BHI, aerobic). *Lb. sakei* No. 7, *Lb. curvatus* No. 15, *Lc. mesenteroides* No. 11, were isolated from spoiled cured meats (Holley, Doyon, Fortin, Rodrigue & Carbonneau, 1996) (all purpose tween broth, APT, anaerobic). *L. monocytogenes,* University of Manitoba, Department of Food Science culture collection (BHI, aerobic). *S. typhimurium* No. 98; AAFC collection, Guelph ON (BHI, anaerobic). *Ser. grimesii* S12, was isolated from cooked roast beef, AAFC collection, Summerland, BC (BHI, anaerobic). *Sh. putrefaciens*, ATCC No. 8071, (BHI, aerobic).

Cultures were maintained monthly on agar slants at 4°C and as frozen glycerol stocks. Cultures were streak-plated once a week and cultures for experiments were inoculated into media from a single colony and incubated overnight under the appropriate atmospheric conditions. All cultures were maintained in either APT or BHI media, except for *Lb. sakei* No. 7 which was maintained on M17 agar, but grown in APT broth . The MI7 medium was used for the maintenance of *Lb. sakei* since the glycerol phosphate it contains prevented rapid reduction in pH.

Organisms were recovered from inoculated meat samples using the following selective media: *B. thermosphacta,* aerobic, streptomycin thallous acetate actidione agar (STAA); *E. coli* O157:H7, aerobic, BHI agar with 30 ppm nalidixic acid (BHI + nal); *Lb. curvatus, Lb. sakei* and *Lc. mesenteroides,* anaerobic, deMan, Rogosa and Sharpe agar (MRS); *L. monocytogenes*, aerobic, modified oxford medium agar (MOX); *S. typhimurium,* anaerobic, brilliant green agar with sulfadiazine (BGS); *Ser. grimesii,* aerobic, violet red bile glucose agar (VRBG); *Sh. putrefaciens,* aerobic, peptone iron agar (PI). In all cases colonies on selective media were enumerated after 48 h incubation at 24°C.

2.2. Materials

Commercially prepared chopped ham mix (12.5% meat protein, pork, water, salt, sugar, dextrose, sodium phosphate, carrageenan, sodium erthorbate, sodium nitrite) and bologna emulsion (pork, mechanically separated turkey, chicken or pork; beef or beef byproducts, water, wheat flour, potato starch, salt dextrose, spices, sodium erythorbate, sodium nitrite, and smoke) were provided by Maple Leaf Meats, Winnipeg, MB. Sausage casings (hog intestine, 30 mm diameter) were from Canada Compound Western, Winnipeg, MB. Sausage casings were stuffed with meat emulsions using a 9 L capacity mechanical sausage stuffer from F. Dick Gmbh, Germany. Low oxygen permeable polyvinylediene chloride (PVDC) plastic bags were kindly provided by Winpak, Winnipeg, MB (25×35 cm, Deli No. 1). Lysozyme:nisin, 1:3 (lot No. 9312-26, 23.75%

(w/w) pure lysozyme, 1.69% (w/w) pure nisin) was premixed and provided by Canadian Inovatech, Abbotsford, BC. EDTA was from Fisher Scientific, Toronto, ON. APT broth, BGS and SMA (standard methods agar) were from BBL, Becton-Dickinson, Franklin Lakes, NJ. BHI broth, granulated agar, MRS broth (Lactobacillus MRS broth), violet red bile bile glucose agar, peptone iron agar, proteose peptone No. 3, oxford medium base, modified oxford antimicrobic supplement (colistin sulfate 10 mg l^{-1}, moxalactam 20 mg l^{-1}) for MOX, and yeast extract were from Difco, Becton-Dickinson. M17 agar was supplied by Oxoid Ltd, Basingstoke, UK. Streptomycin sulphate, cycloheximide and thallous acetate were from Sigma-Aldrich, Oakville, ON. An Autoplater 4000 equipped with a CASBA-4 automated counting system was used for surface plating on pre-poured agar media and enumeration (Spiral Biotech, Inc., Bethesda, MD). The anaerobic incubator (model 3640-6) was from National Appliance Co., Portland, OR, and anaerobic conditions were created by flushing twice with 30% CO_2/70% N_2.

2.3. Methods

The ham and bologna sausages used in these experiments were each prepared from a single batch of chopped ham mix or bologna emulsion. The raw meat batters were obtained from the manufacturer on the day of production and stored at −20°C until the sausages were to be made.

The meat batters were thawed overnight at 4°C and then divided into two equal lots. One lot remained untreated and was used to prepare control samples. The treated samples were prepared by the addition of 500 mg kg^{-1} of lysozyme:nisin, 1:3 and 500 mg kg^{-1} of EDTA, followed by mechanical mixing to ensure homogeneity. Using a 9 l capacity hand-cranked sausage stuffer, treated and control sausages of ham and bologna mix were prepared in the natural casings. The casings were originally salted, but were soaked overnight in deionized water at 4°C to remove the salt. After stuffing to 12 cm lengths, the sausages were clipped with a metal ring, then cooked to an internal temperature of 69°C, packaged and stored at −20°C until inoculated. The heating of the sausages was conducted in a jacketed steam kettle for 3 intervals of 20 min at temperatures of 52.8, 63.9, and 75°C. The sausages were then vacuum packed in heat-sealed O_2 barrier (PVDC) plastic bags and stored at −20°C until required for inoculation. The pH of the cooked sausage was determined by suspending 5 g samples in 45 ml of distilled water.

A 50 ml culture of the organism to be tested was prepared in BHI or APT broth, as appropriate, and incubated aerobically or anaerobically for 48 h at room temperature. A dipping bath for inoculation of the control and inhibititor-treated samples with each test organism was prepared by diluting 1 ml of culture in 1 l of 0.1% peptone, resulting in an initial population of approximately 10^4 CFU cm^{-2} on the meats.

Ham and bologna, control (no inhibitor) and treated sausages were thawed overnight at 4°C and then cut on a sterile board using a flame-sterilized knife into 14 mm "coins". This produced portions of meat 29–31 mm in diameter and 14 mm on a side, with a surface area (maximum) of 28.73 cm^2 and weighing 10 ± 1 g. Three coins were prepared for each sampling group (ham, bologna, control and treatment) and samples for all 5 subsequent time point analyses were prepared simultaneously. The samples were then inoculated by dipping in the inoculum bath, using a 21 cm diameter stainless steel mesh basket for 30 s and then were allowed to dry on sterile filter paper in a laminar airflow hood for 15 min prior to packaging.

Three coins from each sample group were used as time zero samples, held at 4°C and sampled within the next 8 h. The remaining sample units were divided into groups of 3 for each time point and vacuum packed in heat-sealed low O_2 permeable PVDC plastic bags (<15 cm^3/cm^2/day atm 23°C) using a Bizerba model GM 2002 vacuum packaging machine (Mississauga, ON). The samples were then placed at 8°C to incubate for later sampling. Sampling was conducted weekly for 4 weeks after inoculation or until the test organism had reached levels of $\geqslant 10^7$ CFU cm^{-2}.

At each time point the coins from each sampling group were removed from their packages and placed separately in a stomacher bag with 90 ml of 0.1% peptone and massaged (Stomacher 400, A.J. Seward, Canlab, Toronto, ON) for 2 min, to produce a 10^{-1} dilution. The mixed samples were then serially diluted, 100 ul in 9.9 ml 0.1% peptone, to produce dilutions of 10^{-3} and 10^{-5}. The 3 dilutions prepared were then plated, in duplicate, on the appropriate selective medium and SMA using the spiral plater. The selective media were incubated under appropriate conditions (see above) for the individual test organisms to allow for specific enumeration, and the SMA plates were incubated anaerobically to yield total anaerobic numbers (TAN). The number of colony forming units (CFU) per gram of sample were converted to CFU cm^{-2} of sample surface.

2.4. Data treatment

The aim of the analysis of the data generated by this experiment was to determine if the presence of lysozyme, nisin and EDTA in the treated samples resulted in significantly different bacterial populations of the test organism and TAN compared to inoculated control samples without the inhibitors.

The data were first screened to determine if there was a practical difference between the bacterial population

of the control and treated samples at each time point during storage. It was deemed that a practical difference in population levels was present if the difference between the means for the control and treatment was equal to or greater than one log CFU cm^{-2}. A value of one log was chosen for practical significance as differences of one order of magnitude are generally regarded as being of microbial significance (Gill & Baker, 1998; Jarvis, 1989). If the difference between the two means were of practical significance, a two way *t*-test with $\alpha = 0.05$, was conducted to determine whether the difference between the means was statistically significant. For results to have been considered significant, the difference between means of control and treatment met the conditions of both practical and statistical significance.

3. Results

The results of the experiments using *B. thermosphacta* and *Lc. mesenteroides* were interpreted with the knowledge that these were initially packaged under incomplete vacuum . Once this was discovered, the samples were immediately repackaged appropriately under vacuum.

On both control (inoculated but no inhibitor) and treated (with inhibitor) samples, populations of *Lb. sakei*, *Lc. mesenteroides* on ham, *L. monocytogenes* on ham, and *Ser. grimesii* on both meats, rose to exceed 7 log CFU cm^{-2} within the first week of storage at 8°C. No significant difference was observed between bacterial numbers recovered from control and treated samples of ham and bologna inoculated with *Lb. sakei*, *Ser. grimesii* and *Sh. putrefaciens*.

On both ham and bologna samples treated with lysozyme, nisin and EDTA, no *B. thermosphacta* were recovered (< 1.81 log CFU cm^{-2}), up to and including 4 weeks of storage. In contrast, on control samples *B. thermosphacta* populations rapidly increased (Figs. 1 and 2), reaching numbers > 7 log CFU cm^{-2} within one week of storage at 8°C.

On ham, the numbers of *E. coli* O157:H7 remained constant at approximately 4 log CFU cm^{-2} on treated samples, whereas the numbers on control samples increased by 3 log CFU cm^{-2} (Fig. 3). The *E. coli* O157:H7 population on treated bologna dropped by

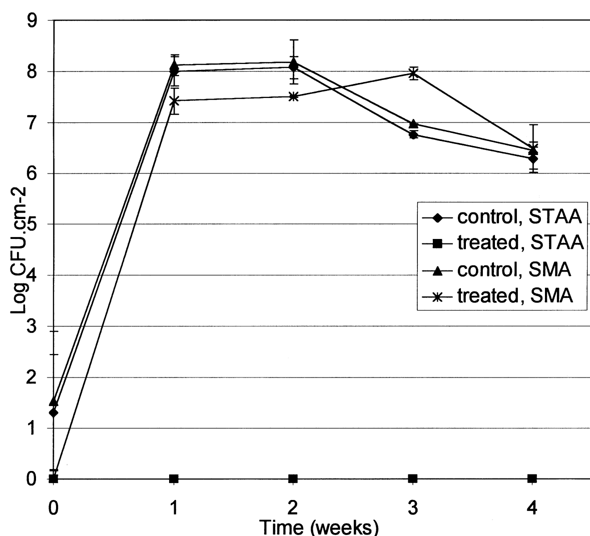

Fig. 1. Growth of *Brochothrix thermosphacta* on ham formulated with lysozyme:nisin, 1:3 (500 mg kg^{-1}) plus EDTA (500 mg kg^{-1}) (treated), or without inhibitor (control). All samples were fully cooked before inoculation with the test organism, vacuum packaged and stored at 8°C. Organisms were recovered on standard methods agar (SMA) or streptomycin thallus acetate actidione agar (STAA). Vertical bars represent one standard deviation interval. Data points without visible vertical bars have a standard deviation of < 0.1 CFU cm^{-2}.

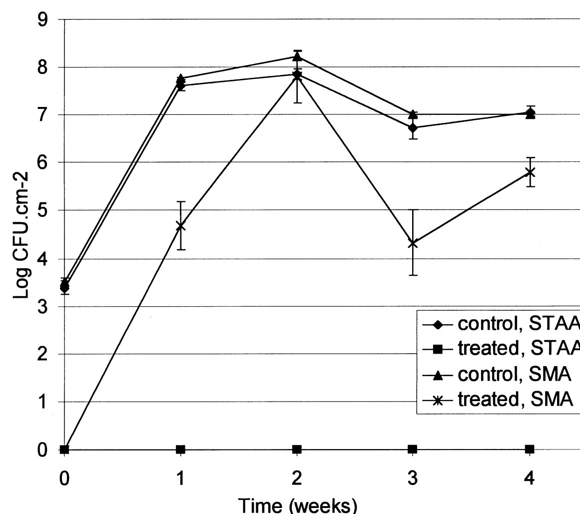

Fig. 2. Growth of *Brochothrix thermosphacta* on bologna formulated, cooked, inoculated, packaged, and stored as described in Fig. 1.

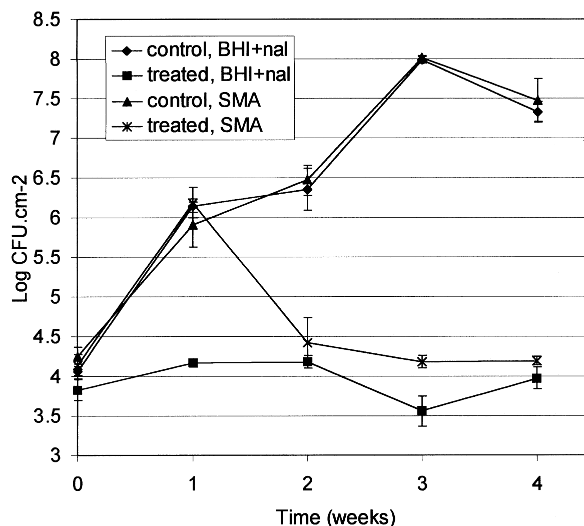

Fig. 3. Growth of *Escherichia coli* O157:H7 on ham formulated with (treated), or without inhibitor (control) as in Fig. 1. All samples were fully cooked before inoculation with *Escherichia coli* O157:H7, vacuum packaged and stored at 8°C. Organisms were recovered on standard methods agar (SMA) or brain heart infusion agar containing 30 ppm nalidixic acid (BHI + nal).

approximately 1 log CFU cm^{-2} over the first 3 weeks of storage before suddenly rising to 8.33 log CFU cm^{-2} by the fourth week. On control bologna the population of *E. coli* O157:H7 rose steadily to 7.03 log CFU cm^{-2} (Fig. 4).

Although initial numbers of *Lb. curvatus* on control and treated meats were not significantly different, subsequent numbers of viable organisms on both ham and bologna were significantly lower on the treated samples, up to and including 3 weeks of incubation. The difference in *Lb. curvatus* population level between control and treated samples on ham was 1 log CFU cm^{-2} at weeks 1 and 2, which increased to 2 log CFU cm^{-2} at week 3 (Fig. 5). On bologna the difference in population levels was approximately 2 log CFU cm^{-2} at weeks 1, 2 and 3 (Fig. 6).

The numbers of *Lc. mesenteroides* recovered from ham was observed to be lower on treated samples (log CFU cm^{-2}, 2.22 sd 0.39) than control samples (log CFU cm^{-2}, 4.75 sd 0.01) at time zero. However, on ham, no difference in bacterial populations was observed between control and treated samples from week one onward. The *Lc. mesenteroides* populations on treated bologna were significantly lower by at least 1 log CFU cm^{-2} up to week 3 of storage (Fig. 7).

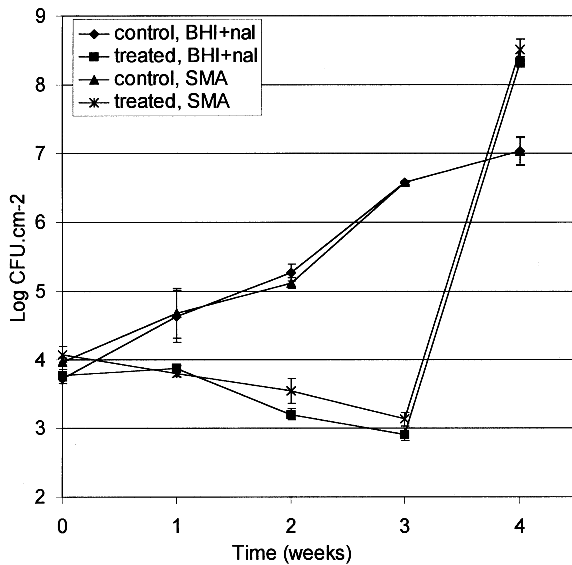

Fig. 4. Growth of *Escherichia coli* O157:H7 on bologna formulated, cooked, inoculated, packaged, and stored as described in Fig. 3.

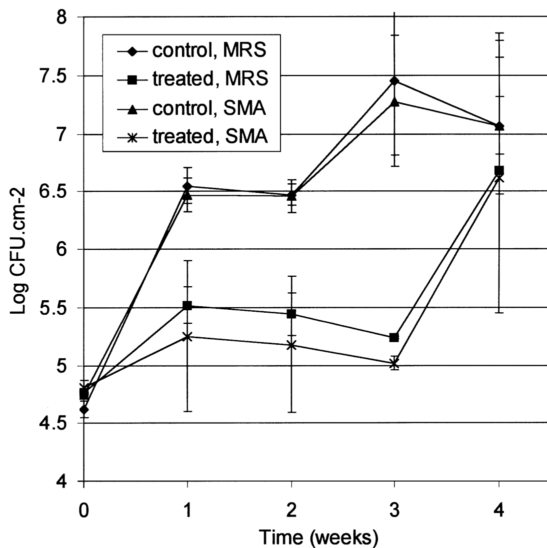

Fig. 5. Growth of *Lactobacillus curvatus* on ham formulated with (treated), or without inhibitor (control) as in Fig. 1. All samples were fully cooked before inoculation with *Lactobacillus curvatus*, vacuum packaged and stored at 8°C. Organisms were recovered on standard methods agar (SMA) or deMan, Rogosa and Sharpe agar (MRS).

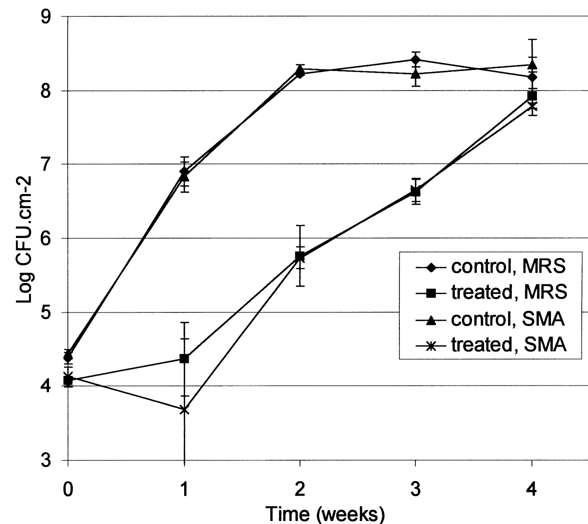

Fig. 6. Growth of *Lactobacillus curvatus* on bologna formulated, cooked, inoculated, packaged and stored as described in Fig. 5.

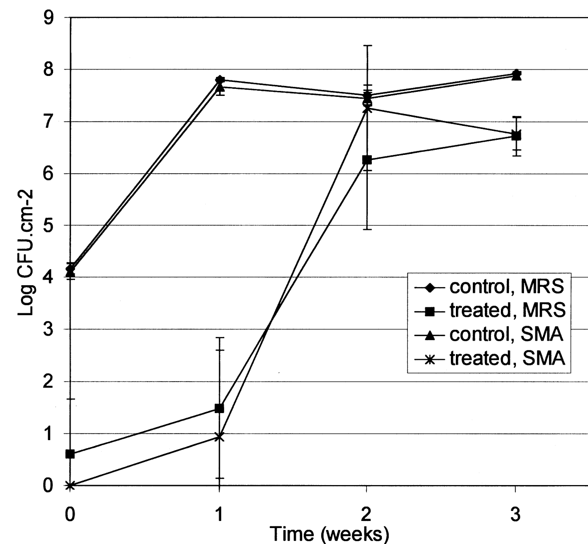

Fig. 7. Growth of *Leuconostoc mesenteroides* on bologna formulated with (treated), or without inhibitor (control) as in Fig. 1. All samples were fully cooked before inoculation with *Leuconostoc mesenteroides*, vacuum packaged and stored at 8°C. Organisms were recovered on standard methods agar (SMA) or deMan, Rogosa and Sharpe agar (MRS).

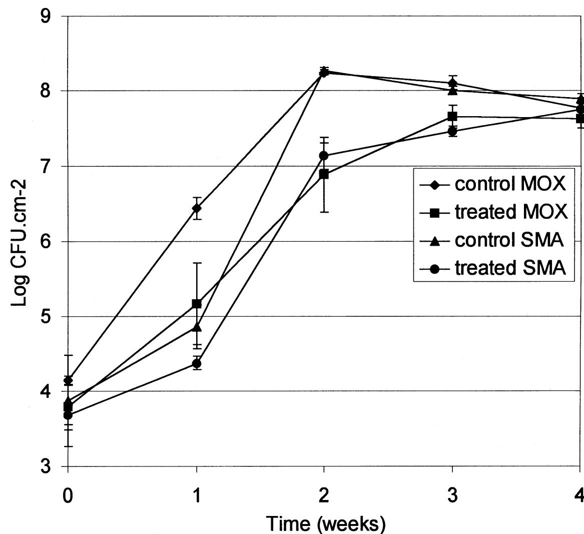

Fig. 8. Growth of *Listeria monocytogenes* on bologna formulated with (treated), or without inhibitor (control) as in Fig. 1. All samples were fully cooked before inoculation with *Listeria monocytogenes*, vacuum packaged and stored at 8°C. Organisms were recovered on standard methods agar (SMA) or modified oxford agar (MOX).

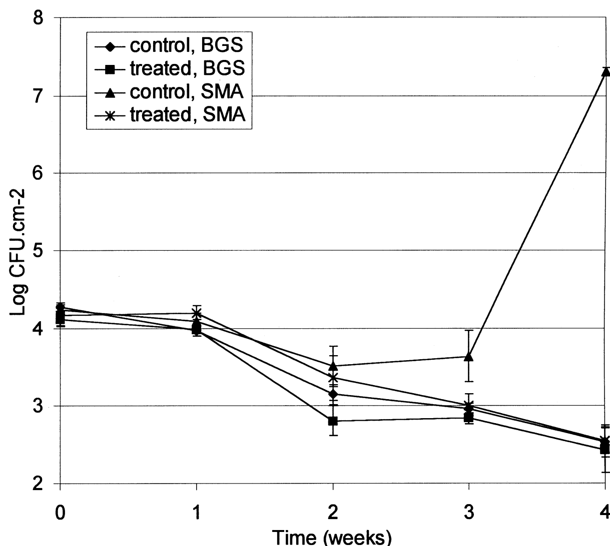

Fig. 9. Growth of *Salmonella typhimurium* on ham formulated with (treated), or without inhibitor (control) as in Fig. 1. All samples were fully cooked before inoculation with *Salmonella typhimurium*, vacuum packaged and stored at 8°C. Organisms were recovered on standard methods agar (SMA) or brilliant green agar with sulfadiazine (BGS).

No significant difference was observed between *L. monocytogenes* populations on control or treated samples of ham. On bologna, the population of *L. monocytogenes* was at least 1 log CFU cm^{-2} lower on treated samples up to two weeks of incubation (Fig. 8).

The results also were taken to indicate that the growth of *S. typhimurium* on treated bologna or ham was not significantly different from controls. The numbers of *S. typhimurium* on both control and treated ham and

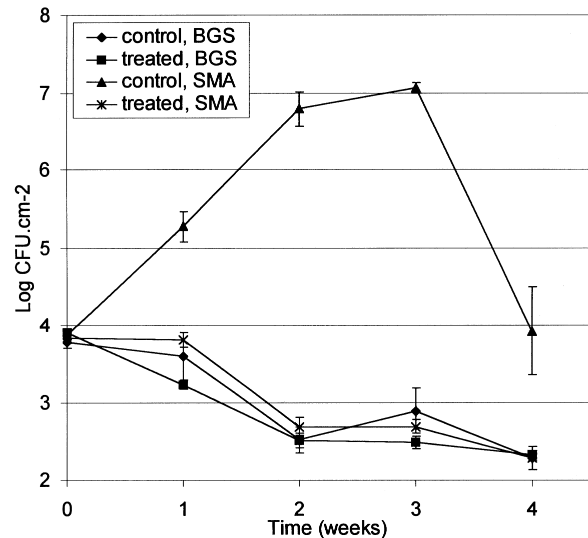

Fig. 10. Growth of *Salmonella typhimurium* on bologna formulated, cooked, inoculated, packaged, and stored as described in Fig. 9.

bologna dropped by 1.5–2 log CFU cm^{-2} between the first week and week 4 (Figs. 9 and 10).

4. Discussion

The results generated from this experiment indicate that the addition of 500 mg kg^{-1} 1:3, lysozyme:nisin and 500 mg kg^{-1} EDTA to ham and bologna mix prior to cooking may restrict the growth of *B. thermosphacta*, *E. coli* O157:H7 and *Lb. curvatus*. Addition of the same antimicrobial agents to bologna also restricted the growth of *Lc. mesenteroides* and *L. monocytogenes*. The presence of the antimicrobials had no apparent effect upon the growth of *Lb. sakei*, *Lc. mesenteroides*, *L. monocytogenes* on ham, or *S. typhimurium*, *Ser. grimesii,* and *Sh. putrefaciens* on either meat. Results obtained with *Lc. mesenteroides* and *L. monocytogenes* inoculated ham as well as those with *Lb. sakei, Ser. grimesii,* and *Sh. putrefaciens* are not presented here. Vacuum packed cooked ham and bologna did not appear to be a favorable environment for *S. typhimurium* as the numbers of this organism decreased on controls over 4 weeks of storage at 8°C.

It was observed that the numbers of *B. thermosphacta* and *Lc. mesenteroides* recovered from both ham and bologna that was treated with inhibitors were lower than those recovered from controls at $T=0$. Since both control and inhibitor treated samples were inoculated from the same source this would indicate that the antimicrobials had a bactericidal effect upon these organisms. The treatment reduced *B. thermosphacta* numbers below detectable levels (<1.81 log CFU cm^{-2}) and inhibited the growth of any survivors for 4 weeks of incubation. The treatment also reduced the initial population of *Lc. mesenteroides* on both ham and

bologna. Though *Lc. mesenteroides* populations recovered within a week on ham, the growth rate on bologna was reduced and it took 2 weeks for populations on inhibitor-treated samples to reach the same levels as controls. The lack of vacuum conditions for the first two days of incubation supports the conclusion that the inhibitor was highly effective against *B. thermosphacta*, as the growth of this organism at cured meat pH is unrestricted under poor vacuum (Borch, Kant-Muermans & Blixt, 1996). However, the poor vacuum conditions may have resulted in an increased growth rate for *B. thermosphacta* on controls and altered the composition of the background flora. For the facultative anaerobe *Lc. mesenteroides*, the lack of a vacuum may have reduced inhibitor effects at week one and altered the composition of the background flora, but this would not have affected the initial population levels.

The lysozyme-nisin treatment appeared to reduce the growth rate of *Lb. curvatus* on both ham and bologna but did not appear to inhibit growth by other LAB. Similar results were obtained with *L. monocytogenes,* though growth was only significantly inhibited on bologna.

The inhibitor treatment reduced the growth of *E. coli* O157:H7 on ham. On bologna, treatment was a little more effective but resulted in only a drop of 1 log CFU cm^{-2} in *E. coli* O157:H7 numbers over the first 3 weeks of storage. This indicates that on bologna the treatment had a lethal effect on *E. coli* O157:H7 cells under vacuum storage. The cause of the sudden rise in the *E. coli* O157:H7 numbers on treated bologna between week 3 and week 4 is unknown. If due to a failed seal on the packaging this could indicate that the treatment was ineffective under aerobic conditions. It would not seem likely that the rise was a result of reduced antimicrobial activity of lysozyme-nisin over time as the same result was not observed on ham.

It was observed in all experiments, except those with *B. thermosphacta* and *E. coli* O157:H7, that the total anaerobic numbers (TAN), from incubation on SMA, closely paralleled the population levels on the selective media. This indicates that, even when inhibited by lysozyme-nisin treatment these organisms still formed the dominant microflora on the product. With *B. thermosphacta* it was observed that TAN from both control and treated samples rapidly rose to spoilage levels on both ham and bologna. This suggests that in the absence of *B. thermosphacta,* other organisms (probably LAB) were able to replace it as the dominant flora. On ham, *S. typhimurium* remained the dominant flora on both control and treated samples, until week 4 when TAN were observed to be significantly greater on controls. On treated bologna *S. typhimurium* formed the dominant flora over the period of storage. However, on inoculated control bologna the population of *S. typhimurium* was consistently less than the TAN.

In a number of the experiments described here it was noted that there was a difference between the activity of the inhibitory treatment against a particular organism on ham as opposed to bologna. The greater effectiveness on bologna may have resulted from the presence or absence of additional restrictive conditions, such as greater residual nitrite levels (stability) or lower water activity (Holley et al., 1996). Our analysis rules out any effect of pH. The pH values of cooked ham and bologna measured in our study (6.52 and 6.49, respectively) were typical of commercial products. Other authors (Cutter & Siragusa, 1997; Murray & Richard, 1997) have observed that nisin activity may be rapidly reduced after addition to meat products. It was speculated that the difference between the effectiveness of the treatment between ham and bologna may result from a difference in the ability of the antimicrobial agents to persist in the product.

The results of these experiments indicate that a lysozyme, nisin and EDTA combination treatment may be of use in controlling the colonization and growth of select organisms of concern in safety and spoilage of cured meat products. Homofermentative LAB and *Leuconostoc* spp. (Holley, 1997b; Yang & Ray, 1994) are most frequently responsible for spoilage of these types of products. Since these experiments were conducted at 8°C to allow rapid evaluation of inhibitory effects, it can be expected that the observed inhibition of microbial growth would be enhanced by incubation at lower temperatures, such as 4°C, which is commonly recommended for storage of cured meat products.

Future research is required to examine the effect of this treatment on a wider range of organisms. Additionally, investigation of alternative treatment delivery systems may be warranted.

The cooking of the product may reduce the activity of treatment agents incorporated into the product. At pH 2 nisin can withstand prolonged heating at 115°C, though stability decreases as pH increases and some food components can be protective. Pasteurization of milk for cheese production does not reduce nisin activity by more than 20% (Delves-Broughton et al., 1996). The enzyme activity of lysozyme can be reduced by pasteurization temperatures, though the extent of inactivation is dependent upon the environment (Proctor & Cunningham, 1988). In contrast, Ibrahim (1998) has shown that the antimicrobial activity of lysozyme may be enhanced by heating though enzymatic activity is reduced.

If heating does reduce antimicrobial activity of the antimicrobial agents then one approach may be to incorporate the treatment agents into a packaging system to be applied to the surface of the product after thermal treatment. This is an attractive alternative since it has been observed that the growth of microbial populations on cured meats occurs on the surface of the

products and colonization results from post-heat treatment contamination (Holley, 1997a,b). Sequestering of the treatment agents in a packaging material or film would localize the agent concentration at the site of microbial activity, which would probably result in more efficient activity against sensitive organisms.

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