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ORIGINAL ARTICLE

Reductions of *Listeria innocua* and *Brochothrix thermosphacta* on beef following nisin spray treatments and vacuum packaging

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Sections of UV sterilized lean and adipose tissues from the surfaces of post-rigor (24 h post-mortem) beef carcasses were inoculated with Brochothrix thermosphacta *or* Listeria innocua *to obtain approximately 4·50 log_{10} cfu cm^{-2} and subjected to spray treatments with sterile water or nisin (5000 AU ml^{-1}). Untreated and spray treated samples were vacuum-packaged, and incubated at 4°C for up to 4 weeks. Bacterial populations from untreated vacuum-packaged tissues and spray treated, vacuum-packaged tissues were enumerated on non-selective and selective media at 0, 7, 14, 21 or 28 days. Nisin spray treatments of lean and adipose vacuum-packaged tissues reduced the numbers of* L. innocua *up to 2·83 log_{10} cfu cm^{-2}. Additionally, nisin sprays and vacuum packaging effectively suppressed* L. innocua *during the 4-week incubation such that the remaining bacteria did not grow to the same level as untreated or water-treated, vacuum-packaged tissues. Nisin spray treatments and vacuum packaging of lean and adipose tissues reduced* B. thermosphacta *to undetectable levels. Data from this study demonstrate that nisin spray treatments followed by vacuum packaging under refrigerated conditions could increase the shelf life by suppressing or inhibiting the growth of undesirable bacteria present on fresh beef.*

Introduction

Several reports have addressed the use of bacteriocins to effectively suppress the growth of bacteria on, or in, various processed red meat products (Bell and DeLacy 1986, Calderon et al. 1985, Houben and Krol 1985, Rayman et al. 1981, Taylor et al. 1985, Taylor and Somers 1985, Chung et al. 1989, Berry et al. 1990, Nielsen et al. 1990, Schillinger et al. 1991, Degnan and Luchansky 1992, Degnan et al. 1992, 1993, Foegeding et al. 1992). In our laboratories, we have demonstrated that nisin spray treatments of post-rigor beef resulted in immediate reductions of approximately 2 log_{10} cfu cm^{-2} for *Listeria innocua* and up to 3 log_{10} cfu cm^{-2} for *Brochothrix thermosphacta* and *Carnobacterium divergens*, as well as suppression of the organisms during a 24 h aerobic incubation at 4°C (Cutter and Siragusa

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1994a). In another study, nisin dip treatments, followed by bacterial inoculation and modified atmosphere packaging storage at 4°C were effective for preventing *L. monocytogenes* growth on cooked pork, compared with untreated controls (Fang and Lin 1994).

Vacuum packaging is commonly used to extend the shelf life of perishable foods, including most fresh, unfrozen, red meats. Approximately 93% of all beef leaving commercial slaughterhouses in the United States is vacuum-packaged (personal communication, Charles Jolley, Cryovac Division, W. R. Grace & Co., Duncan, SC). Although there are numerous benefits associated with vacuum packaging of red meat under refrigerated conditions, spoilage by lactobacilli and *B. thermosphacta* (Egan and Grau 1981, Gardner 1981, Qvist and Mukherji 1981) and growth of listeriae do occur (Gill and Reichel 1989, Grau and Vanderlinde 1990, Buncic et al. 1991). No studies have documented the efficacy of bacteriocin spray treatments and vacuum packaging under refrigerated conditions for reducing bacterial populations on fresh beef, to our knowledge. Therefore, the following study was carried out to determine if a combination of nisin spray washes followed by vacuum-packaged storage could be used to extend the shelf life and/or improve the microbiological safety of fresh, refrigerated beef.

Materials and Methods

Bacterial cultures

Brochothrix thermosphacta ATCC 11509 and *Listeria innocua* LA1 (Roman L. Hruska U. S. Meat Animal Research Center (RLHUSMARC) culture collection) were maintained in 75% glycerol at –20°C. *L. innocua* was propagated in trypticase soy broth supplemented with 0·5% yeast extract (TSBYE; Troy Biologicals, Troy, MI) at 37°C for 18 h; *B. thermosphacta* was propagated in TSBYE at 25°C for 18 h.

Inoculation of beef

Lean and adipose tissues from the outer surfaces of post-rigor (24 h post-mortem) beef carcasses were obtained from RLHUSMARC abattoir, trimmed to $7{\cdot}5 \times 7{\cdot}5 \times 0{\cdot}5$ cm, surface sterilized by UV light, and stored at –20°C, as described previously (Cutter and Siragusa 1994b). Sterility was monitored by individually sampling three pieces of lean or adipose tissue at day 0 using the enumeration procedures described below. Overnight (18 h) cultures of *L. innocua* and *B. thermosphacta* were diluted 1:100 in sterile physiological saline (pH 7·0) to obtain a viable cell population of approximately 9 $\log_{10}$ cfu ml^{-1}. After thawing to 23°C, the fascia of individual pieces of lean and adipose tissues were placed in a sterile weigh boat (14×14 cm) containing 10 ml of the bacterial suspension, incubated for 15 min, 25°C and allowed to drip for 15 s. Inoculated samples were left untreated or subjected to spray treatments with water or nisin. Bacterial populations of approximately 4·50 $\log_{10}$ cfu cm^{-2} were obtained using this procedure.

Bacteriocin preparation and activity

Purified nisin (Ambicin™, Applied Microbiology, NY) was solubilized in distilled water, filter sterilized (0·2 µm Vacucap™, Gelman Sciences, Ann Arbor, MI), and added to sterile distilled water for a final concentration of 5000 activity units/ml (pH 6·0), (as determined by the manufacturer) and used immediately after preparation. Spot assays were performed to monitor nisin activity (Siragusa 1993). In our laboratories, well-diffusion assays indicated that *L. innocua* was more resistant to nisin than *L. monocytogenes* Scott A (unpublished data). Because of the risk of exposure to the pathogen during spraying, *L. innocua* was used as a pathogen model in our study.

Bacteriocin spray treatments and experimental design

A pilot scale model carcass washer located at RLHUSMARC was used to apply water or nisin as described previously (Cutter and Siragusa 1994a,b). Operation parameters for

the carcass washer were as follows: spray nozzle oscillation speed, 80 cycles min^{-1}; chain speed, 14 m min^{-1}; line pressure, 60 psi; flow rate, 4·2 l min^{-1}; nozzle distance from sample, 17·8 cm; temperature of solutions, 28°C (Cutter and Siragusa 1994a,b). Immediately after spray treatments with either water or nisin, a 25 cm^2 section was aseptically excised from the spray treated tissues, placed into a Sterefil™ Stomacher bag (Spiral Biotech, Bethesda, Maryland), the top folded over, and stored at 4°C until processed or vacuum-packaged. Day 0 samples were prepared for bacterial enumeration within 1 hr after spray treatment. The remaining 25 cm^2 sections of untreated and spray treated tissues in Stomacher bags were vacuum-packaged (>26 in mmHg; Vacusave, Tilia, Inc., San Francisco, CA) according to the manufacturer's instructions. The oxygen transmission rate of the nylon/olefin/polyethylene bags was <2·6 cc 100 in^{-2} 24 h^{-1} at 25°C and 0% relative humidity; moisture vapor transmission was <0·7 Gr 100 in^{-2} 24 h^{-1} at 100°C and 90% relative humidity. All samples were incubated at 4°C and sampled for bacterial populations at 7, 14, 21 or 28 days. Controls consisted of lean and adipose tissues that were inoculated with bacteria for 15 min, excised, transferred to a sterile Stomacher bag, vacuum-packaged, stored, and sampled at 7, 14, 21 or 28 days. After excising the 25 cm^2 section, remaining pieces of untreated and spray treated tissues were put into sterile Whirl-Pak™ bags (Nasco, Fort Atkinson, WI), vacuum-packaged as described above and used to assess surface pH values (flat electrode, Corning Instruments, Corning, NY) at day 0 and after storage at 4°C for 7, 14, 21 or 28 days.

Bacterial enumeration

At 0, 7, 14, 21 or 28 days of refrigeration, each 25 cm^2 piece of untreated or spray treated, vacuum-packaged tissue was pummeled for 2 min (Stomacher 400, Tekmar, Inc., Cincinnati, OH) in the Stomacher bag with 50 ml of buffered peptone water (BPW, pH 7·0; BBL, Cockeysville, MD) with 0·1% Tween 20 (Fisher; St. Louis, MO). Each stomachate was serially diluted in BPW, and spiral plated (Model D Spiral Plater; Spiral Biotech, Bethesda, MD) in duplicate on trypticase soy agar (TSA; Difco, Detroit, MI) supplemented with 0·5% yeast extract (Difco; TSAYE). Stomachates from samples inoculated with *L. innocua* were also spiral plated onto lithium chloride phenylethyl alcohol (LPM) agar (BBL, Cockeysville, MD) and stomachates containing *B. thermosphacta* were spiral plated onto STAA agar (Oxoid, Hampshire). Plates were enumerated after incubation for 36 h at 37°C for *L. innocua* and for 36 h at 30°C for *B. thermosphacta*. Enzymatic inactivation of nisin was not included in the experimental design of this study because residual nisin did not interfere with plating procedures in the previous study (Cutter and Siragusa 1994a).

Calculations and statistical analyses

The experiment was 2 (organisms) × 2 (tissue types) × 3 (treatments) × 5 (days) factorially arranged and completely randomized. After enumeration, bacterial populations were converted to cfu cm^{-2}. Separate statistical analyses were performed on bacterial populations enumerated from non-selective (TSAYE) and selective (STAA, LPM) media used in this study. Least-squared means (LSM) of bacterial populations (log_{10} cfu cm^{-2}) were calculated from three experimental replications. Analysis of variance (ANOVA) was performed using the General Linear Models (GLM) procedure of SAS (SAS Institute, ver. 6·06·01, 1989, SAS Inst., Inc., Cary, NC, 1982). Inoculum counts were used as a covariate to normalize data between treatment replications. Log_{10} cfu cm^{-2} reductions were calculated at each day as differences between LSM of untreated tissues and LSM of nisin- or water-treated tissues (LR = LSM log_{10} cfu cm^{-2} untreated–LSM log_{10} cfu cm^{-2} treated). LSM values were calculated from three replications of surface pH data for both tissue types at each day of sampling. ANOVA was performed using the GLM procedure of SAS. The probability level for population or surface pH data was $P \leq 0{\cdot}05$, unless otherwise noted.

Results

Spray treatments and vacuum packaged data from non-selective media

From the data for bacterial populations grown on the non-selective media, TSAYE, there were no significant four-way or three-way interactions (Table 1). A significant two-way interaction of day × tissue as well as the individual effects of treatment, tissue, and day were observed. Analysis of the overall treatment effect on total bacterial populations grown on TSAYE indicated that spray treatments with nisin resulted in a population of 4·23 $\log_{10}$ cfu cm^{-2} which was significantly different than untreated or water-treated populations of 6·92 and 6·46 $\log_{10}$ cfu cm^{-2}, respectively. Overall analysis of the tissue effect on bacteria grown on TSAYE demonstrated that bacterial populations on adipose tissues were significantly higher (6·51 $\log_{10}$ cfu cm^{-2}) than lean tissues (5·23 $\log_{10}$ cfu cm^{-2}) over the 4-week incubation period. Analysis of the day effect demonstrated that populations at days 0 (3·48 $\log_{10}$ cfu cm^{-2}) and 7 (5·54 $\log_{10}$ cfu cm^{-2}), were significantly different from each other and were both different from days 14 (6·55 $\log_{10}$ cfu cm^{-2}), 21 (6·64 $\log_{10}$ cfu cm^{-2}) or 28 (7·15 $\log_{10}$ cfu cm^{-2}), which were statistically similar.

Untreated, water- or nisin-spray treated bacterial populations, including *L. innocua* on adipose and lean tissues over the 4-week incubation period and enumerated on TSAYE, are listed in Figs 1(a), 1(b). Initial (day 0) LRs of 2·70 and 2·30 were observed for bacteria on adipose and lean tissues, respectively following nisin spray treatments. Populations of nisin-treated bacteria were consistently lower than either untreated or water-treated bacteria throughout the 4-week incubation, regardless of day or tissue.

Data from untreated, water- or nisin-treated bacterial populations, including *B. thermosphacta*, on lean or adipose tissues and enumerated on TSAYE, are presented in Figs 2(a) and 2(b). As demonstrated previously (Cutter and Siragusa, 1994a), nisin spray treatments of bacteria resulted in initial LR of 2·54 and 2·36 $\log_{10}$ cfu cm^{-2} on adipose and lean tissues, respectively. The data also indicated that although populations of bacteria increased over the course of incubation, none of the populations associated with nisin spray treatments reached the level(s) of untreated or water-treated bacteria.

Data for samples enumerated on TSAYE also demonstrated that initially (day 0), water spray treatments resulted in LR of <1 $\log_{10}$ cfu cm^{-2} on lean or adipose tissue. However, suppression of the organisms was not sustained by water-spray treatments because bacterial levels were comparable with

Table 1 Analysis of variance of data obtained from samples enumerated on TSAYE

Interaction	Degrees of freedom	Pr>F
Treatment	2	0·0001
Organism	1	0·3715
Tissue	1	0·0001
Day	4	0·0001
Treatment × organism	2	0·0654
Treatment × tissue	2	0·9437
Organism × tissue	1	0·2631
Treatment × day	8	0·9102
Organism × day	4	0·4885
Tissue × day	4	0·0323
Treatment × tissue × day	8	0·9715
Organism × tissue × day	4	0·1641
Treatment × organism × day	8	0·9187
Treatment × organism × tissue	2	0·7827
Treatment × organism × tissue × day	8	0·4064

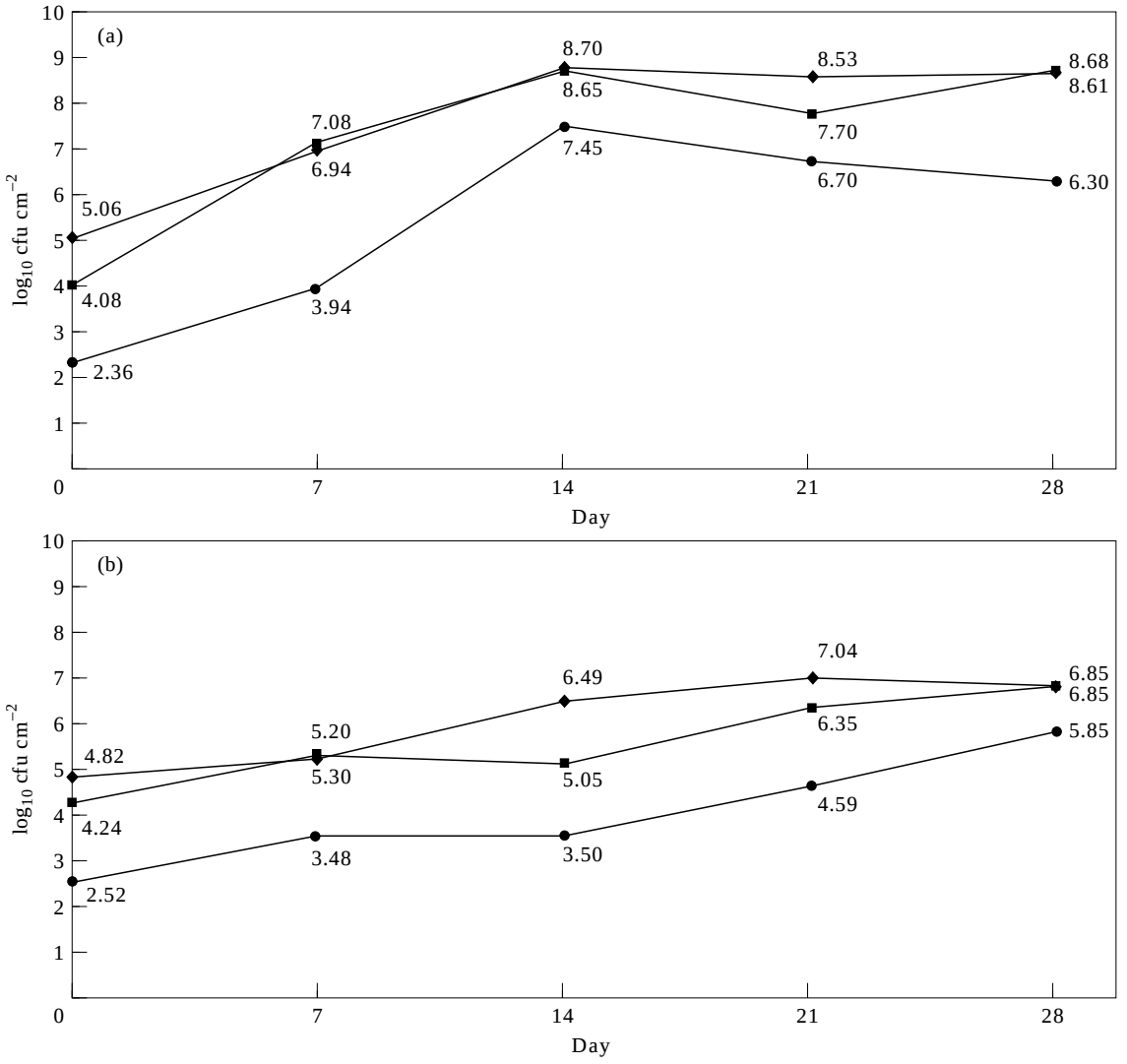

Figure 1. Following spray treatments with water or nisin (5000 AU ml^{-1}) and vacuum packaging, bacterial populations, including *Listeria innocua* LA1, attached to adipose (a) or lean (b) tissues were enumerated at days 0, 7, 14, 21 and 28 on TSAYE. Least square means (LSM) of bacterial populations from three replications are presented at each point. (◆), untreated; (■), water; (●), nisin.

untreated populations by the end of the experiment.

Spray treatments and vacuum packaged data for selective media

Although lean and adipose tissues were surface sterilized by UV light, completely sterile conditions could not be maintained after spray treatments or vacuum packaging procedures. Therefore, all stomachates containing either *L. innocua* or *B. thermosphacta* were also plated on LPM and STAA, respectively. Overall analysis of the bacterial populations from the selective media demonstrated a significant three-way interaction of treatment × organism × day and two-way interactions of treatment × organism, and organism × day (Table 2). All of the main effects (treatment, organism, tissue and day) were also found to be statistically significant. Bacterial populations of either *L. innocua* or

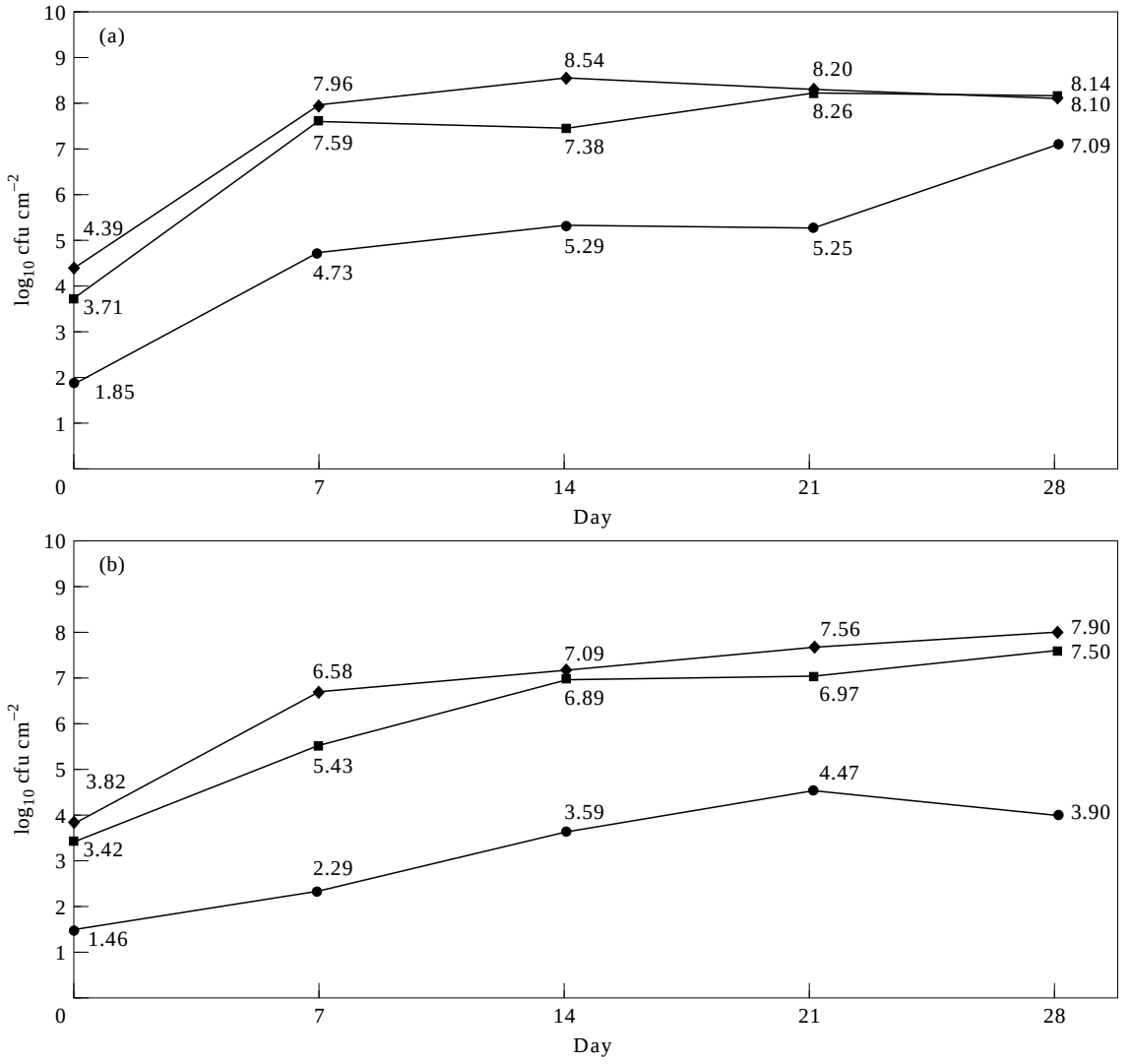

Figure 2. Following spray treatments with water or nisin (5000 AU ml^{-1}) and vacuum packaging, bacterial populations, including *Brochothrix thermosphacta* ATCC 11509, attached to adipose (a) or lean (b) tissues were enumerated at days 0, 7, 14, 21 and 28 on TSAYE. Least square means (LSM) of bacterial populations from three replications are presented at each point. (◆), untreated; (■), water; (●), nisin.

B. thermosphacta on nisin-treated tissues (2·48 log_{10} cfu cm^{-2}) were significantly lower than untreated (6·85 log_{10} cfu cm^{-2}) or water-treated (6·49 log_{10} cfu cm^{-2}) tissues. Populations of *B. thermosphacta* were significantly lower (5·04 log_{10} cfu cm^{-2}) than *L. innocua* (5·51 log_{10} cfu cm^{-2}). Surviving bacterial populations were also greater on adipose tissues (5·78 log_{10} cfu cm^{-2}) than lean tissues (4·77 log_{10} cfu cm^{-2}). Populations of either *L. innocua* or *B. thermosphacta* at day 0 (3·57 log_{10} cfu cm^{-2}) were statistically different than days 7 (5·12 log_{10} cfu cm^{-2}) and 14 (5·32 log_{10} cfu cm^{-2}), which were statistically different than days 21 (5·99 log_{10} cfu cm^{-2}), or 28 (6·38 log_{10} cfu cm^{-2}).

As was demonstrated by enumeration on TSAYE, bacterial populations of *L. innocua* on adipose and lean tissues subjected to nisin spray treatments were initially reduced by 2·83 and 2·58 log_{10} cfu cm^{-2}, respectively. However, *L. innocua* was effectively sup-

Table 2 Analysis of variance of data obtained from samples enumerated on selective media

Interaction	Degrees of freedom	Pr>F
Treatment	2	0·0001
Organism	1	0·0132
Tissue	1	0·0001
Day	4	0·0001
Treatment × organism	2	0·0001
Treatment × tissue	2	0·0681
Organism × tissue	1	0·0965
Treatment × day	8	0·0995
Organism × day	4	0·0096
Tissue × day	4	0·2849
Organism × tissue × day	4	0·6545
Treatment × tissue × day	8	0·3268
Treatment × organism × day	8	0·0022
Treatment × organism × tissue	2	0·8824
Treatment × organism × tissue × day	8	0·8433

Table 3 Analysis of variance of data obtained from surface pH values

Interaction	Degrees of freedom	Pr>F
Treatment	2	0·0075
Organism	1	0·0796
Tissue	1	0·0001
Day	4	0·0001
Treatment × tissue	2	0·2436
Organism × tissue	1	0·6665
Treatment × organism	2	0·9544
Treatment × day	8	0·7051
Organism × day	4	0·0201
Tissue × day	4	0·0001
Treatment × organism × tissue	2	0·4214
Treatment × organism × day	8	0·7544
Treatment × tissue × day	8	0·4984
Organism × tissue × day	4	0·1572
Treatment × organism × tissue × day	8	0·5739

pressed by nisin on adipose tissues only over the 4-week incubation under vacuum-packaged and refrigerated conditions (Fig. 3(a)). Remaining populations of *L. innocua* on lean tissues eventually did grow to levels similar to untreated or water treated tissues (Fig. 3(b)).

At day 0, nisin spray treatments initially reduced populations of *B. thermosphacta* by 4·67 $\log_{10}$ cfu cm^{-2} on adipose tissues and 3·12 $\log_{10}$ cfu cm^{-2} on lean tissues, as indicated by the data presented in Figs 4(a) and 4(b). When combined with vacuum packaging, nisin spray treatments effectively reduced *B. thermosphacta* on adipose and lean tissues to $\leq$1·3 $\log_{10}$ cfu cm^{-2}, with the result that *B. thermosphacta* was not detected for the duration of the experiment.

Surface pH data

Statistical analysis of surface pH data demonstrated two-way interactions of organism × day and tissue × day as well as significant treatment, day and tissue effects. Analysis of the treatment effect of surface pH demonstrated that untreated tissues (5·90) were not statistically different from water-treated tissues (5·97), but were statistically different from nisin-treated tissues (6·04). The day

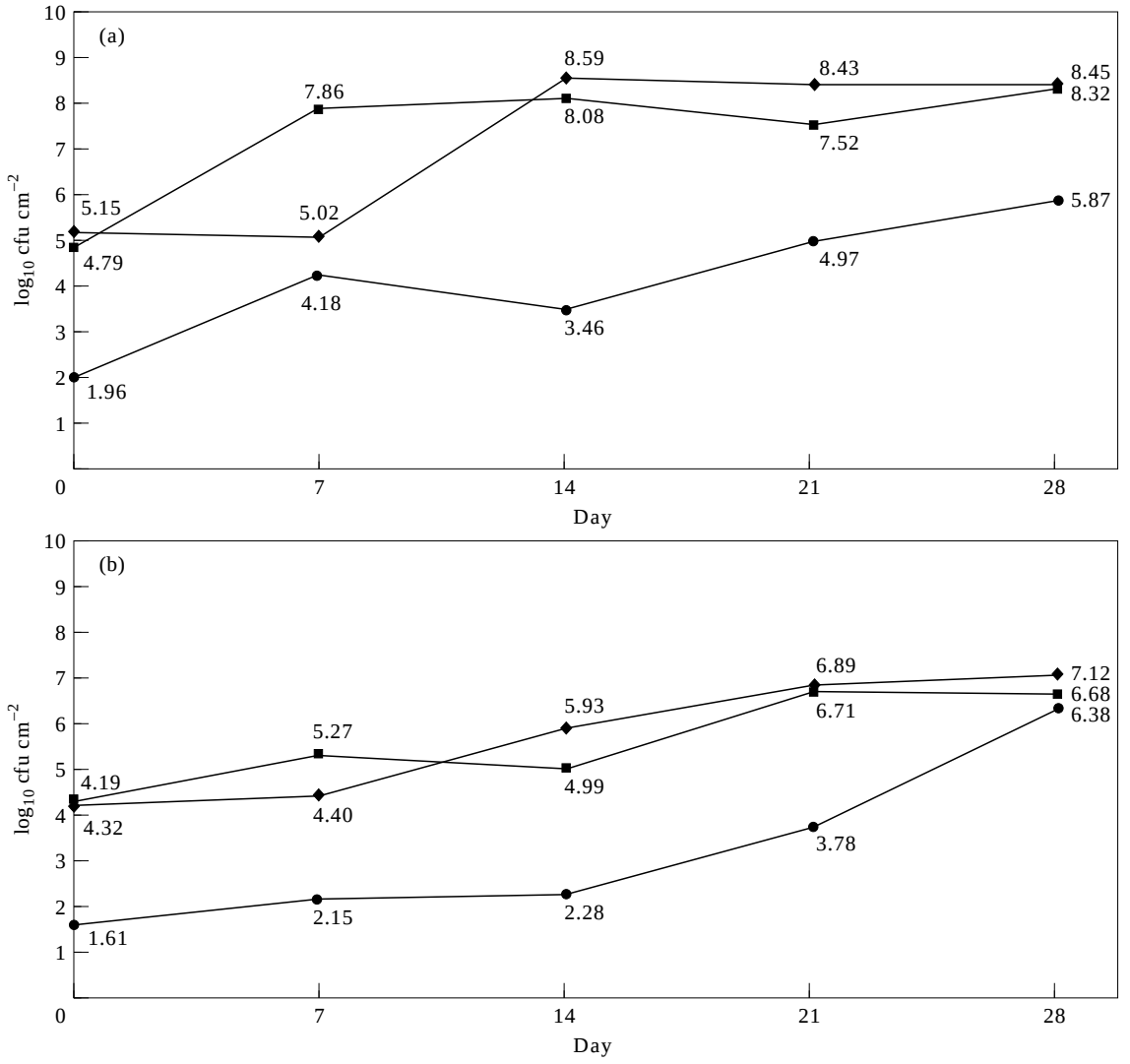

Figure 3. Following spray treatments with water or nisin (5000 AU ml^{-1}) and vacuum packaging, populations of *Listeria innocua* LA1 attached to adipose (a) or lean (b) tissues were enumerated at days 0, 7, 14, 21 and 28 on LPM. Least square means (LSM) of bacterial populations from three replications are presented at each point. (◆), untreated; (■), water; (●), nisin.

effect demonstrated that pH values at day 0 (6·21) were significantly different than at days 7 (5·97), 14 (5·89), 21 (5·87) and 28 (5·92). Analysis of tissue effect indicated that the surface pH of adipose tissue (6·37) was significantly different than lean tissue (5·58).

Discussion

It is known that vacuum packaging increases the shelf life of red meat products by either suppressing the growth of some spoilage bacteria by oxygen deprivation or inhibition by carbon dioxide (Gill 1986). However, both *B. thermosphacta* and listeriae are psychrotrophic, Gram-positive organisms capable of growth on the surfaces of pork, lamb, poultry, fish and beef under vacuum-packaged conditions. *B. thermosphacta* is the predominant spoilage organism of vacuum-packaged or modified atmosphere packaged meat (Gill 1986, Jay 1986), and pathogenic listeriae are a concern to public health.

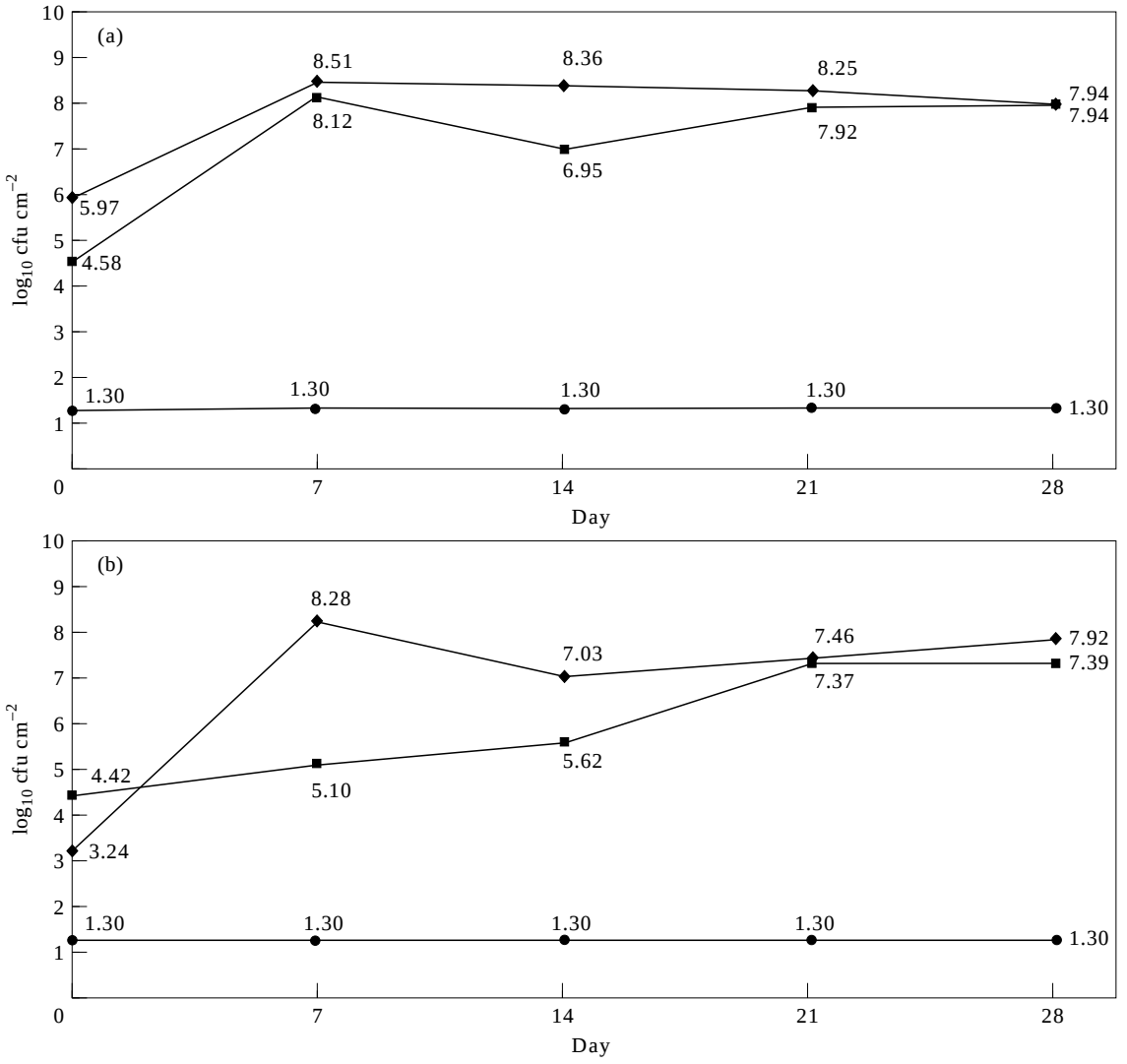

Figure 4. Following spray treatments with water or nisin (5000 AU ml^{-1}) and vacuum packaging, populations of *Brochothrix thermosphacta* ATCC 11509 attached to adipose (a) or lean (b) tissues were enumerated at days 0, 7, 14, 21 and 28 on STAA. Least squares means (LSM) of bacterial populations from three replications are presented at each point. (◆), untreated; (■), water; (●), nisin.

As has been demonstrated previously (Cutter and Siragusa 1994a) and in this study, nisin spray treatments initially reduced bacterial populations greater than 99%, for the organisms tested. The growth of organisms on non-selective media following initial spray treatments can be attributed to the presence of nisin-resistant contaminants, or the recovery and growth of sublethally injured listeriae. Despite differences associated with the various parameters (tissue type, day, organism) examined in this study, nisin spray treatments followed by vacuum packaging were efficacious for reducing populations of *B. thermosphacta* and *L. innocua* on fresh beef. *L. innocua* was used as a pathogen model in this study because of its greater resistance to nisin, compared with *L. monocytogenes* (unpublished data). Our results suggest that *L. monocytogenes* associated with fresh beef may be reduced by similar nisin sprays and vacuum packaging treatments. Nisin spray treatments may not only improve the shelf life of vacuum-packaged beef by

inhibiting the growth of spoilage bacteria such as *B. thermosphacta*, but may also enhance the microbiological safety of beef by reducing levels of pathogenic bacteria.

In our earlier study (Cutter and Siragusa 1994a), spray treatments with water or nisin did not significantly alter the pH of the lean or adipose tissues at days 0 or 1. Therefore, population reductions were attributed to the inhibitory activity of nisin and not to the effects of pH and/or spray washing (Cutter and Siragusa 1994a). In this study, nisin was applied at pH 6·0 to minimize inhibition due to pH changes. However, surface pH values did differ throughout the experiment. The surface pH of adipose tissues was greater than lean tissues; this observation is in agreement with other published studies (Dickson 1992, Cutter and Siragusa 1994b). Bacterial growth on beef is known to result in formation of numerous compounds that may affect surface pH (Stanley et al. 1981, Gill 1986, Jay 1986). We demonstrated that pH values of tissues subjected to nisin sprays were shown to be statistically higher than untreated tissues. Because suppression of bacterial growth may result in fewer acidic metabolites, reduced bacterial growth by nisin sprays may result in higher pH values, as seen in this study. Conversely, the reduced surface pH of untreated and water-treated tissues over the 4-week incubation may have resulted from bacterial growth and possible formation of other metabolites. Additional surface pH differences observed in this study may be attributed to the formation of carbonic acid and/or other compounds produced by bacteria during vacuum packaging (Stanley et al. 1981, Gill 1986, Jay 1986).

We have demonstrated that a combination of nisin spray treatments and vacuum packaging under refrigerated conditions is effective for reducing undesirable bacteria on red meat. Such a combination may be useful as a multi-hurdle approach to improve the microbial stability and ultimately, the shelf life and safety of red meat.

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References

Bell, R. G. and DeLacy, K. M. (1986) Factors influencing the determination of nisin in meat products. *J. Food Technol.* **21,** 1–7.

Berry, E. D., Liewen, M. B., Mandigo, R. W. and Hutkins, R. W. (1990) Inhibition of *Listeria monocytogenes* by bacteriocin-producing *Pediococcus* during the manufacture of fermented semidry sausage. *J. Food Protect.* **53,** 194–197.

Buncic, S., Paunovic, L. and Radisic, D. (1991) The fate of *Listeria monocytogenes* in fermented sausages and in vacuum-packaged frankfurters. *J. Food Protect.* **54,** 413–417.

Calderon, C., Collins-Thompson, D. L. and Usborne, W. R. (1985) Shelf life studies of vacuum-packaged bacon treated with nisin. *J. Food Protect.* **48,** 330–333.

Chung, K.-T., Dickson, J. S. and Crouse, J. D. (1989) Effects of nisin on growth of bacteria attached to meat. *Appl. Environ. Microbiol.* **55,** 1329–1333.

Cutter, C. N. and Siragusa, G. R. (1994a) Decontamination of beef carcass tissue with nisin using a pilot scale model carcass washer. *Food Microbiol.* **11,** 481–489.

Cutter, C. N. and Siragusa, G. R. (1994b) Efficacy of organic acids against *Escherichia coli* O157:H7 attached to beef carcass tissue using a pilot scale model carcass washer. *J. Food Protect.* **57,** 97–103.

Degnan, A. J. and Luchansky, J. B. (1992) Influence of beef tallow and muscle on the antilisterial activity of pediocin AcH and liposome-encapsulated pediocin AcH. *J. Food Protect.* **55,** 552–554.

Degnan, A. J., Yousef, A. E. and Luchansky, J. B. (1992) Use of *Pediococcus acidilactici* to control *Listeria monocytogenes* in temperature-abused vacuum-packaged wieners. *J. Food Protect.* **55,** 98–103.

Degnan, A. J., Buyong, N. and Luchansky, J. B. (1993) Antilisterial activity of pediocin AcH in model food systems in the presence of an emulsifier or encapsulated within liposomes. *Int. J. Food Microbiol.* **18,** 127–138.

Dickson, J. S. (1992) Acetic acid action on beef tissue surfaces contaminated with *Salmonella typhimurium*. *J. Food Sci.* **57,** 297–301.

Egan, A. F. and Grau, F. H. (1981) Environmental conditions and the role of *Brochothrix thermosphacta* in the spoilage of fresh and processed meat. In *Psychrotrophic Microorganisms in Spoilage and Pathogenicity* (Eds Roberts, T. A., Hobbs, G., Christian, J. H. B. and

Skovgaard, N.) pp. 211–221. London, Academic Press.

Fang, T. J. and Lin, L.-W. (1994) Growth of *Listeria monocytogenes* and *Pseudomonas fragi* on cooked pork in a modified atmosphere packaging/nisin combination system. *J. Food Protect.* **57,** 479–485.

Foegeding, P. M., Thomas, A. B., Pilkington, D. H. and Klaenhammer, T. R. (1992) Enhanced control of *Listeria monocytogenes* by in situ-produced pediocin during dry fermented sausage production. *Appl. Environ. Microbiol.* **58,** 884–890.

Gardner, G. A. (1981) *Brochothrix thermosphacta* (*Microbacterium thermosphactum*) in the spoilage of meats: a review. In *Psychrotrophic Microorganisms in Spoilage and Pathogenicity* (Eds Roberts, T. A., Hobbs, G., Christian, J. H. B. and Skovgaard, N.) pp. 139–173. London, Academic Press.

Gill, C. O. (1986) The control of microbial spoilage in fresh meats. In *Advances in Meat Research, Vol. 2, Meat and Poultry Microbiology* (Eds Pearson, A. M. and Dutson, T. R.) pp. 49–88. Westport, Connecticut, AVI Publishing Co., Inc.

Gill, C. O. and Reichel, M. P. (1989) Growth of cold-tolerant pathogens *Yersinia enterocolitica, Aeromonas hydrophila* and *Listeria monocytogenes* on high-pH beef packaged under vacuum or carbon dioxide. *Food Microbiol.* **6,** 223–230.

Grau, F. H. and Vanderline, P. B. (1990) Growth of *Listeria monocytogenes* on vacuum-packaged beef. *J. Food Protect.* **53,** 739–741.

Houben, J. H. and Krol, B. (1985) Controlling growth of *Streptococcus faecium* in a ham model with heat and ethylendiamine tetracetic acid, tertiary butylhydroquinone or nisin. *Meat Sci.* **13,** 205–215.

Jay, J. M. (1986) *Modern food microbiology*, 3rd edn. New York, Van Nostrand Reinhold Co.

Nielsen, J. W., Dickson, J. S. and Crouse, J. D. (1990) Use of bacteriocin produced by *Pediococcus acidilactici* to inhibit *Listeria monocytogenes* associated with fresh meat. *Appl. Environ. Microbiol.* **56,** 2142–2145.

Qvist, S. and Mukherji, S. (1981) *Brochothrix thermosphacta*—an important spoilage agent in vacuum-packaged sliced meat products. In *Psychrotrophic Microorganisms in Spoilage and Pathogenicity*. (Eds Roberts, T. A., Hobbs, G., Christian, J. H. B. and Skovgaard, N.) pp. 223–230. London, Academic Press, Inc.

Rayman, M. K., Aris, B. and Hurst, A. (1981) Nisin: a possible alternative or adjunct to nitrite in the preservation of meats. *Appl. Environ. Microbiol.* **41,** 375–380.

Schillinger, U., Kaya, M. and Lucke, F.-K. (1991) Behaviour of *Listeria monocytogenes* in meat and its control by bacteriocin-producing strain of *Lactobacillus sake*. *J. Appl. Bacteriol.* **70,** 473–478.

Siragusa, G. R. (1993) Production of bacteriocin inhibitory to *Listeria* species by *Enterococcus hirae*. *Appl. Environ. Microbiol.* **58,** 3508–3513.

Stanley, G. K., Shaw, J. and Egan, A. F. (1981) Volatile compounds associated with spoilage of vacuum-packaged sliced luncheon meat by *Brochothrix thermosphacta*. *Appl. Environ. Microbiol.* **41,** 816–818.

Taylor, S. L. and Somers, E. B. (1985) Evaluation of the antibotulinal effectiveness of nisin in bacon. *J. Food Protect.* **48,** 949–952.

Taylor, S. L., Somers, E. B. and Krueger, L. A. (1985) Antibotulinal effectiveness of nisin-nitrite combinations in culture medium and chicken frankfurter emulsions. *J. Food Protect.* **48,** 234–239.