# Use of Pediocin With Other Barriers For Control of *Listeria monocytogenes* on Ready-to-Eat (RTE) Processed Meats

Project Report to

American Meat Institute Foundation

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**Project Objectives**:

The general objectives of this project were to; 1) study effectiveness of pediocin AcH for control of *Listeria monocytogenes* (Lms), 2) determine effectiveness of combined barriers such as pediocin with post-packaging thermal treatments, and pediocin with post-packaging irradiation treatments, and 3) evaluate delivery systems for pediocin including product spraying, coextrusion processing and interior coatings for casings.

**Procedures**:

Pediocin selection

Preliminary efforts for this project began with production of pediocin from *Pediococcus acidilactici* strain H, utilizing the Iowa State University Fermentation Laboratory. The organism was grown in MRS Lactobacillus broth supplemented with yeast extract. Cells were killed by exposure of the culture to 75º C. Pediocin was separated from the cells and removed by centrifugation. The pediocin preparation was filter-sterilized and frozen until use.

The activity of the pediocin preparation was assayed by plating *Listeria monocytogenes* on tryptic soy broth with 0.6% yeast extract (TSBYE). Serial dilutions of the pediocin preparation were placed on the surface of the plates, which were then incubated at 37º C. Plates were observed for inhibition zones greater than 2 mm in diameter on the indicator lawn. The inhibitory strength was determined and expressed in arbitrary units (AU). One AU was defined as the inverse of the highest dilution of pediocin that produced an inhibition zone of 2-mm diameter or more.

Initial small-scale production of pediocin resulted in high levels of activity but subsequent large-scale production attempts were less successful. The culture of *Pediococus acidilactici* showed reduced production of the pediocin. Replacement of the culture with a new culture yielded similar results. Because the pediocin activity obtained from the large-scale production efforts was similar to that of the commercially available Alta 2341® (Quest International), it was decided to use Alta 2341® as a source of pediocin for this study. Using the commercial product assured a dependable supply of pediocin with a consistent level of activity. Further, by using a commercial product, results from this study can be easily reproduced and used by the industry or by other researchers.

Frankfurter preparation:

Frankfurters were manufactured from frozen beef trim (~80% lean) and fresh pork trim (~50% lean) purchased from commercial suppliers. Frozen trim was removed from the freezer (-20º C) 24 hours before processing and tempered at 2º C to 4º C. All trim was coarse ground (0.95-cm plate) and fat content measured by Anyl-Ray (Kartridg-Pak Co., Davenport, IA). Frankfurter batches were formulated with a 45.4 kg (100 lbs) meat block plus 9.1 kg ice/water, 1.49 kg commercial spice mixture (A.C. Legg Packing Co., Inc., Birmingham, AL), 112 g curing salt (AC, Legg Packing Co., Inc., Birmingham, AL) and 908 g salt.

The lean meat was chopped first with ice/water, salt, spices and cure to about 4.5º C after which the fat trim was added and chopping completed to 13.9º C to form the batter. The frankfurter batter was vacuum stuffed (Risco Model RS 4003-165; Stoughton, MA) into 24 mm cellulose casings and linked at 12 to 14 cm in length. Stuffed frankfurters were smoked, cooked

and showered in an Alkar oven (Alkar, Lodi, WI). Chilling was achieved in a 2º C cooler overnight before peeling. Finished frankfurters were chilled for 12 to 18 hours before random assignment to different experimental treatments. Finished weight of the frankfurters was 45 ±5 g per link.

Fat, moisture (AOAC, 1990) and protein content (combustion method, Model FP-428, LECO Corp., St. Joseph, MI) of the finished products were measured with results indicating the frankfurters contained 28.25% crude fat, 53.66% moisture and 13.44% protein.

Inoculation and packaging

Frankfurters were packaged in three different packaging arrangements as follows; 1) 10 links per package in a double row of 5 each, 2) 5 links per package in a single row, and 3) singly packaged links. Each packaging group was divided into two subgroups for addition of one of two levels of pediocin (Alta 2341® Quest Int., Savasota, FL) before inoculation with *Listeria monocytogenes* (LMS). Alta 2341® was formulated in sterile, distilled water to achieve 3000 arbitrary units (AU) of pediocin per ml. The frankfurters were placed in bags, then sprayed with either 1 or 2 ml per link of the pediocin to result in application of 3000 or 6000 AU per link. The vacuum bags (Cryovac ® B-540, Cryovac Sealed Air Corp., Duncan, SC) with frankfurters and pediocin were hand-massaged for 5 to 10 seconds for even distribution of the pediocin before vacuum sealing (Multivac A 300/52, Mutivav Sepp Haggenmüler GmbH & Co., Wolfertschwendn, Germany). For inoculated samples, 1 ml of a 5-strain cocktail of Lms was added to the packages just prior to vacuum sealing. In preliminary trials with dye, the combination of spraying, massaging and vacuum packaging resulted in uniform distribution across the surface of frankfurters within the package.

For inoculated packages, 5 strains of Lms cultures, including Lms Scott A 5/15, ½ aH7764 5/15, 4b H7769, 4 b H7962 and OB 90393 were individually grown in trypticase soy broth containing 0.6% yeast extract (TSB-YE) at 35º C for 24 hours. After 24 h., 1 ml of each culture was transferred to 500 ml TSB-YE broth and held at 35º C for 24 h to give the 5-strain mixture. The final concentration of the 5-strain cocktail mixture was 9.3 log CFU/ml as determined by previous growth curve experiments.

Inoculation of samples with the 5-strain Lms cocktail was done in similar fashion to the approach described earlier for addition of Alta 2341®. The 5-strain cocktail was first diluted to give either 3 log CFU/ml or 5 log CFU/ml, and then l ml of the cocktail was added per package. The bags were massaged for 5 to 10 secs to distribute the inoculation, and closed under vacuum. After packaging, all samples were placed in paperboard boxes and stored at 2º C to 4º C for 14 to 18 h. before sampling. Recovery of Lms from untreated samples showed inoculated products to have 3.4 and 5.2 CFU/g, respectively for the two inoculation levels.

For post-packaging thermal pasteurization treatments, samples were inoculated as described prior to heat treatment. Pasteurization treatments included water temperatures of 71º C, 81º C or 96º C, each for 30, 60 or 120 s. A water bath (Fisher Isotemp 220) and Fisher immersion circulator (Model 730, Fisher Scientific, Pittsburgh, PA) were used to control water temperature. Packages were held in heated water for the prescribed time, then immersed in 15º C water for 10 min. to chill before placement in refrigerated storage at 2º C to 4º C.

For irradiation pasteurization, inoculated samples were treated with 1 kGy or 2kGy of irradiation using a single- or double-sided irradiation treatment at the linear accelerator facility (Linear Accelerator-Circle III R, Saint-Aubin, France) at Iowa State University. The actual measured average of irradiation doses (minimum and maximum doses) was 1.2 (0.93-1.71) and 2.3 (1.82-3.37) kGy for single-pass treatments and 1.4 (1.29-1.53) and 3.5 (2.54 and 6.40) kGy for double-pass treatments, respectively. The double-pass treatment was necessary for the double-row packages of frankfurters because of the package thickness. The actual delivered doses were measured by placing alanine pellet dosimeters (Bruher Instruments, Inc. Billerica, MA) on the top and bottom of packages for each treatment (ASTM, 1996). Untreated samples served as controls and to check the recovery of Lms.

Evaluation during storage

Based on initial evaluations, treatments that showed the greatest immediate effectiveness were selected for subsequent evaluation of long-term effectiveness during storage. Treatments selected included Alta 2341® in combination with post-packaging thermal pasteurization at 81º C for 60 s. and 96º C for 60 s and 120 s, and Alta 2341® in combination with irradiation. All package variations (single, 5 and 10 links per package) were included. Inoculations for the storage evaluation were 3 log CFU/g of the Lms cocktail. Storage of samples was at 4º C, 10º C and 25º C for up to 12 weeks.

Microbiological Analyses

Initial evaluations. Packages of frankfurters were aseptically opened, using sterile scissors, 18 h after treatments. Except for single- link packages, samples were collected by taking two frankfurters, one from the center of the package row and one from the outside. Frankfurters were aseptically cut in half (20-23 g portions) with sterile scissors and tweezers. Samples were homogenized (Seward Stomacher blender, Model 4000, Tekmer Co., Cincinnati, OH) for two minutes in sterile stomacher bags (Whirl-Pak® Filter Bag B01318, A Nasco, Ft. Atkinson, WI) containing 0.1% sterile peptone water in the amount to give a 1 to 5 dilution of the sample. Lms cells were enumerated by serially diluting l ml of the blended samples in 9 ml of 0.1% peptone water, plating on Modified Oxford agar (MOA) and Trypticase Soy Agar (TSA) and incubating at 35º C for 48 h. Typical colonies were enumerated, identified by gram stain and confirmed using api Listeria kits (bioMérieux, Inc., Hazelwood, MO). For samples heated with Alta 2341® and irradiation, TSA with yeast extract (YE) was used for plating to improve recovery of Lms.

Enumeration during storage. For enumeration of samples held in storage, packages were opened aseptically and whole frankfurters were mixed for 1 min in a Seward Stomacher blender with an equal amount of 0.1% peptone water (50% dilution) to rinse them thoroughly. Lms cells were enumerated by serial dilutions of the rinse in 9 ml of 0.1% peptone water, plating on MOA and TSA-YE and identified as described previously.

Physical and Chemical Analyses of Frankfurters

After completion of the microbiological evaluations, the treatments that had the most impact on Lms were also evaluated for product quality changes. Frankfurters were prepared, processed and treated in the same way as for the inoculation challenge, but without the Lms

inoculation. Quality evaluations included measurement of purge accumulation, color, texture, odor quality, pH and thiobarbituric acid (TBA) values.

For purge, two packages (5 links per package) were each weighed, opened and the frankfurters removed. The package and links were wiped dry and reweighed. The weight difference was calculated as purge and expressed as a percentage of product weight (Bloukas et al., 1997).

Color (L, a, b) measurements utilized a Hunter Labscan spectrophotometer (Hunter Associated Laboratories, Inc., Reston, VA), using illuminant A and 10° C observer (incandescent light) with a 0.25 in. port insert. Samples were overwrapped with clear saran film and color measured at two locations (center and end) of each frankfurter. Five links from each treatment were measured for color. For texture measurement, a Texture Analyzer (Model TA-XT 2I, Godalming, U.K.) was used for assessment of skin toughness and interior firmness by measuring puncture resistance and interior texture with a 3-mm puncture probe. Five frankfurters from each treatment were measured in the center and the end of each link. The probe was programmed to penetrate 12 mm into the samples following measurement of the surface skin resistance. Penetration speed was 1.5 mm/second. All samples were measured at room temperature 3 h. after removal from refrigeration.

For pH measurement, 10 g of sample was blended with 90 ml of distilled water in a Waring Blendor® and the slurry measured with a pH meter (Fisher Accumet Model 925, Fisher Scientific, Pittsburgh, PA) using a sealed combination electrode (Omega Engineering, Inc., Stamford, CT). The TBA values were measured using the modified method for cured meats. Duplicate measurements of pH and TBA values were recorded for each sample.

Sensory evaluation

The sensory evaluation was conducted using a panel of 15-16 experienced panelists, all being students, staff or faculty in the Department of Food Science and Human Nutrition at Iowa State University. All panelists were volunteers and were trained by using commercial frankfurters, some of which were irradiated, to familiarize the panelists with the properties to be measured and scales to be used. Only uninoculated samples were used for sensory evaluations. Panelists evaluated samples for purge, color, texture, and odor.

Samples were scored using a 15 cm unstructured line scale and were viewed in fluorescent-lighted booths. The amount of purge and exterior color was evaluated using intact, unopened packages of frankfurters. For texture assessment, the panelists used a fork to cut through the center of a frankfurter section. For odor evaluation, frankfurters were heated in boiling water for 2 minutes, cut into sections and placed in 150 ml beakers covered with aluminum foil before presentation to the panel. Panelists evaluated odor for smoky, burnt and acid notes; all of which were established during the training sessions. The numerical scales used for purge, color, odor and texture, were described as; 0 = none, extremely light, none and extremely soft, respectively; while 15 = extremely abundant, dark, intense, and firm, respectively. All sensory evaluations were conducted within two weeks of processing and were repeated three times.

Statistical Analyses

Microbiological data were transformed into logarithms of the number of colony-forming units ($log_{10}$CFU/g). The Statistical Analysis System (SAS, 1993) was used to determine means, standard errors and variance analyses from the three replications. When analysis of variance (ANOVA) revealed a significant difference ($P<0.05$), treatment means were compared using the least significant difference (LSD) test.

Initial evaluations

Data were treated as a split, split-plot design with Lms inoculation level and package size as the main plot, pediocin concentration as the split plot, and temperature x time and irradiation doses as the split-split plot.

All data were analyzed using SAS with the general linear model (GLM) procedure. Comparisons of means were based on Tukey's range test for least significance difference.

Evaluation during storage

The experimental design for the evaluations during storage was a split plot with 13 treatments in the main plot while the subplot consisted of 13 sampling dates from 0 weeks to 12 weeks. All data were analyzed using SAS with the general linear model (GLM) procedure. Comparisons of means were based on Tukey's range test for least significant differences.

**Results**

The experimental protocol was designed to first compare the effects of pediocin concentration, post-packaging thermal pasteurization, irradiation pasteurization, and package arrangements on inhibition of *Listeria monocytogenes* on frankfurters. The most effective combinations were then evaluated for inhibitory effects during storage at 4º C, 10º C and 25º C. The potential to deliver pediocin to product surfaces by using pretreated casings or co-extruded casings was also evaluated. Finally, the most effective treatments were assessed for product quality changes.

Inhibitory Effects of Pediocin (Alta 2341®)

The effects of pediocin concentrations (3000 AU and 6000 AU) are shown in Fig. 1 for the three packaging arrangements (1, 5 or 10 links per package). Both log 3 and log 5 inoculation levels are also included. The results indicate that the pediocin has a significant ($P<0.001$) inhibitory effect which is concentration dependent. Samples inoculated with Lms at log 5 showed a reduction of about 1.5 log and about 2.0 log for the 3000 AU and 6000 AU pediocin levels, respectively. The 3-log inoculations showed reductions of about 1.0 log and 1.5 log respectively for the 3000 AU and 6000 AU pediocin treatments.

Post Packaging Thermal Pasteurization

The exposure of inoculated packages to 71º C, 81º C or 96º C for 30s, 60s, or 120s resulted in a wide range of inactivation effectiveness (Fig. 2, Fig. 3). With a log 5 inoculation (Fig. 2), all treatments resulted in at least a 2-log reduction of Lms. The singly packaged links showed nearly a 5-log reduction of Lms at the higher temperature and the effect was more complete with the greater concentration of pediocin. Both 60s and 120s at 96º C in combination with 6000 AU of pediocin resulted in a full 5 log reduction of Lms. The temperature increments each achieved an additional 1-2 log reduction, as did the time increments at each temperature.

Packages with 5 links showed about a 3-log reduction of Lms at the most effective temperatures. Time effects at each temperature were significant but less marked than for singly packaged frankfurters. The effect of time and of pediocin concentration was again significant but none of the treatments achieved a full 5-log reduction of Lms.

Packages with 10 links per package showed a Lms reduction of about 2.5 logs at best. The impacts of increasing temperature, increasing time and increasing pediocin concentration were all lessened by this package arrangement.

Clearly, the interfacial area between frankfurters that is not in contact with the package film provides for greater survival of Lms during post-packaging thermal pasteurization.

For packages inoculated with Lms at log 3 (Fig. 3), effects were, in general, similar to those observed with a 5-log inoculation. Singly packaged links showed essentially a 3-log reduction except for the 30s treatments at 71º C and 81º C. Thus, 12 of the 18 treatment combinations shown in fig. 3 reduced Lms to undetectable levels for singly-packaged frankfurters.

The packages with 5 links each showed a Lms reduction of about 1.5 log to 2.5 log with increased effectiveness when pasteurization temperature or time was increased. Pediocin concentration was again significant and was more effective at the higher concentration of pediocin used.

The reduction of Lms was between 1.0 log and 2.0 log for packages with 10 links per package. There is less impact of increasing pasteurization time or temperature than observed for the other packaging arrangements.

There were no significant ($P>0.05$) interactions between pediocin concentration and pasteurization temperatures or times and no synergistic effect of these treatments was obvious. The impact of increased pasteurization temperature was generally greater than the impact of increased pasteurization time within the limits of this study.

Irradiation

The results of irradiation treatments, which measured 1.2 kGy and 2.3 kGy of actual delivered doses, are shown in Fig. 4. For the log 3 inoculation of Lms, the 2.3 kGy dose reduced Lms to undetectable levels, regardless of pediocin concentration. For samples irradiated with the 1.2 kGy dose, the reduction was about 2 log without pediocin but was increased to a full 3 log by either concentration of the pediocin. For the log 5 inoculation treatments, pediocin also clearly potentiates the effects of irradiation. At the 2.3 kGy dose, Lms was reduced to 0 when 2 ml (6000 AU) of pediocin was used. The addition of pediocin was significant ($<0.0001$) but the two concentrations used were not different from each other despite the consistently lower values observed for Lms numbers when pediocin concentration was increased. The greater apparent impact of irradiation on the 10-link package is due to a greater total absorbed dose, which occurred because these packages were irradiated on two sides.

Thus, post-packaging irradiation achieved a significant pasteurization effect which was dose-dependent and which was potentiated by the presence of pediocin. There was a significant ($P<0.0001$) interaction between irradiation and addition of the pediocin. The presence of pediocin also appears to result in a synergistic effect on Lms when combined with irradiation.

Results During Storage at 25º C

The growth of Lms in the presence of the pediocin at 25º C is shown in Fig. 5. The organism was inoculated at log 3 and showed an immediate increase in number after 1 day in control samples without pediocin. Addition of pediocin induced about a 1-day lag phase to Lms growth. There was no significant ($P>-0.05$) difference between the two concentrations of pediocin.

Combining the pediocin with post-packaging thermal pasteurization resulted in greater effectiveness during storage at 25º C (Fig. 6). In this case, there is a delay in Lms growth of about 2 days except for the least extreme pasteurization. For the 81º C /60s. treatment, Lms numbers were reduced but growth begins immediately and reaches the control numbers by 5 days. The other treatments, except for the 96º C/120s, reached control numbers after 6-7 days. The 96º C/120s treatment suppressed growth for 8 days and numbers remain lower throughout.

Combining the pediocin with irradiation treatments (Fig. 7) demonstrated the interaction of these treatments even with subsequent storage at 25º C. Irradiation had a very significant effect with the 2.3 kGy treatment resulting in a 6-day delay in growth. Growth is more erratic at different sampling points probably due to damage and injury to Lms cells by the irradiation treatment. The 10-link packages showed virtually complete inactivation of Lms due to the somewhat higher irradiation dose delivered by the double-pass treatment necessary for these packages. The effectiveness of irradiation in combination with pediocin was greater than that of thermal pasteurization in combination with pediocin.

Results During Storage at 10º C

In Fig. 8, the results of storage at 10º C are shown for the two pediocin treatments. Again, as at 25º C, there was no difference between the 3000 AU and the 6000 AU concentrations of pediocin. However, the lag time for growth was about two weeks at 10º C as opposed to 1-2 days at 25º C. After 3 weeks, Lms numbers in the pediocin-treated samples reached those of the controls and remained similar for the rest of the storage period. Results at 10º C were similar to those observed at 25º C except for the longer lag time for Lms growth.

Combinations of thermal pasteurization with pediocin prior to storage at 10º C are shown in Fig. 9. Again, there was about a 2-week lag phase for treatments with pediocin. The most significant effect was observed for the single-packaged franks exposed to 96º C for 120s. These samples did not show growth for 3 ½ weeks, then demonstrated low, erratic recoveries for the remainder of the storage period.

The impact of irradiation combined with pediocin was again evident in samples held at 10º C (Fig. 10). In this case, only the 1.2-kg dose showed consistent growth after a lag phase of 2 weeks. All other treatments suppressed growth over the full 12 weeks of storage at 10º C. Recoveries were erratic suggesting extensive damage to cells by irradiation. Again, the effect of irradiation was greater than that of thermal pasteurization and interactive effects between pediocin and irradiation occurred.

Results During Storage at 4º C

When samples were held at 4º C, pediocin treatments had a significant effect in lowering the Lms counts over the full 12 weeks of storage (Fig. 11). While, on the graph, growth appears to increase after 7-9 weeks, there were no significant ($P>0.05$) differences among the different sampling times. Again, as at 25º C and 10º C, there were no differences between the two concentrations of pediocin used.

The addition of thermal pasteurization treatments to the pediocin treatments resulted in a significant decrease in Lms numbers as expected (Fig. 12). In this case, the lag phase is clearly 7 weeks for the 81º C/60s treatment and 7-10 weeks for the others with the exception of the single-packaged franks treated with 96º C /120s. The latter showed no growth over the 12-week storage period. The 96º C temperature treatment was significantly ($P<0.05$) more effective than the others and temperature had a greater effect than time for thermal pasteurization. For treatments with irradiation and pediocin, the synergistic combination had very marked effects when samples were stored at 4º C (Fig. 13). All treatments resulted in virtually no Lms counts for 12 weeks except for the 1.2 kGy dose. The latter showed recovery and growth after seven weeks.

Storage Temperature Effects

Overall effects of the antilisterial treatments subjected to the three holding temperature can be summarized as follows; pediocin treatments had significant effects in reducing Lms numbers and introducing a lag time for growth. While the initial reduction in Lms numbers depended on the addition of pediocin, the length of the subsequent lag phase was dependent on the temperature. These were no differences due to pediocin concentration at any of the temperatures used. Thermal pasteurization effects were, as noted earlier, very dependent upon packaging arrangement with effectiveness of singly-packaged >5 links per package >10 links per package. Temperature of the post packaging pasteurization, particularly at 96º C, was more important than time in the pasteurization conditions studied. Irradiation was synergistic with the pediocin treatment and, at 4º C, the 2.3 kGy dose was sufficient for the combination to reduce Lms to undetectable levels for 12 weeks.

Product Quality Analyses

Based on the microbiological results, the most effective treatment combinations were evaluated for potential product quality changes that might be introduced by the treatments. The treatments selected for sensory evaluation included the addition of pediocin at both levels (3000 AU and 6000 AU), thermal pasteurization at 81º C and 96º C, each for 60s and irradiation at both doses (1.2 kGy and 2.3 kGy) with 6000 AU of pediocin.

The results for purge, color, texture, pH and oxidative change (TBA values) are presented in table 1. There was no effect of treatments on package purge except for the volumes of solutions added to packages. The addition of pediocin, for example, at 1 ml or 2 ml resulted in a significant difference in the water measured as purge. Most importantly, the thermal pasteurization treatments did not increase the amount of measured purge over that of packages that did not receive a post-packaging heat treatment.

Purge was significantly affected by package arrangement as might be expected with the greater number of frankfurters per package resulting in greater purge. To further explore the effects of thermal treatments on product purge, frankfurters in all three package types, but without added pediocin or inoculum solutions, were submitted to the 81º C and 96º C treatments. The results are shown in Fig. 14. There was no significant effect of the thermal pasteurization conditions on the amount of purge in any of the package types. Package type, however, as pointed out earlier, affected the amount of purge observed.

Color values were modified by the thermal pasteurization treatments and by irradiation. In both cases, the pasteurization treatments resulted in color which was darker (L value) and redder (a value). These differences are not large but were statistically significant and were confirmed by sensory panel evaluations.

Textural characteristics were not affected by any of the product treatments, either for exterior skin toughness or for interior firmness. Product pH or TBA were not affected by the treatments.

Sensory panel evaluations

The sensory panels evaluated frankfurters for visual purge, color, texture and specific preselected aroma notes. The results are shown in Fig. 15. The purge assessment reflects the volume of added solutions such as the 1ml (3000 AU) or 2 ml (6000 AU) of pediocin. The control was significantly ($P<0.0001$) lower for apparent purge than the treatments because there were no added solutions. There was no significant ($P<0.05$) difference in purge as a result of the thermal pasteurization treatments.

Color scores (Fig. 15) were lower for the control group compared with all treatments but this was not significant except for the irradiated treatment group. The irradiated frankfurters, however, were not different from any other treatments other than the control.

Odor scores, evaluated as smoky, burnt and acid aromas, did not differ. Panelist training included irradiated frankfurters, and the odor notes and terminology were developed in the training sessions with irradiated and heat-repasteurized products. Panelists evaluated products that were exposed to a range of heat and irradiation treatments to generate odor notes that might be detected in the experimental products. The experimental treatments did not result in significant changes in product odors.

The texture assessments by the sensory panel indicated increased force was necessary to cut the frankfurters with a fork in the case of the thermal pasteurization treatments (Fig. 15). Irradiation treatments are also higher than the controls for the texture assessment but not significantly so. The addition of pediocin increased the product resistance to slicing and was significant at the higher level of addition (6000 AU). This effect was surprising and a potential explanation is not clear. Instrumental evaluations of texture, however, showed no significant differences among the treatments or between the treatments and the controls for either skin toughness or interior firmness of the frankfurters (table 1).

Finally, two different systems which might serve as carriers for pediocin and other inhibitors were evaluated. For the first, commercial cellulose casings which have an interior coating of either pediocin or pediocin plus sodium diacetate were used for frankfurter manufacturing. After peeling, the frankfurters were packaged and inoculated with log 3 Lms. This treatment resulted in a small reduction of Lms compared to the control but the difference was not significant (table 2). This is, however, an extremely easy system to use and, if effectiveness can be increased, would offer an easy delivery system. The second system evaluated for delivery of the pediocin was a coextrusion of collagen dough with the frankfurter emulsion batter to form an exterior casing. Equipment used for this experiment was the Townsend Kontura (Townsend Engineering, Des Moines, IA) system. Pediocin (40% solution) was blended with the collagen dough during the co-extrusion process. Results from co-extruded, inoculated frankfurters showed a small, but insignificant reduction of Lms numbers, similar to results with the cellulose casings lined with pediocin. The co-extrusion process, however, offers potential for manipulating the amounts of pediocin or other compounds added and may have potential which could be developed with further investigations.

Summary and Conclusions

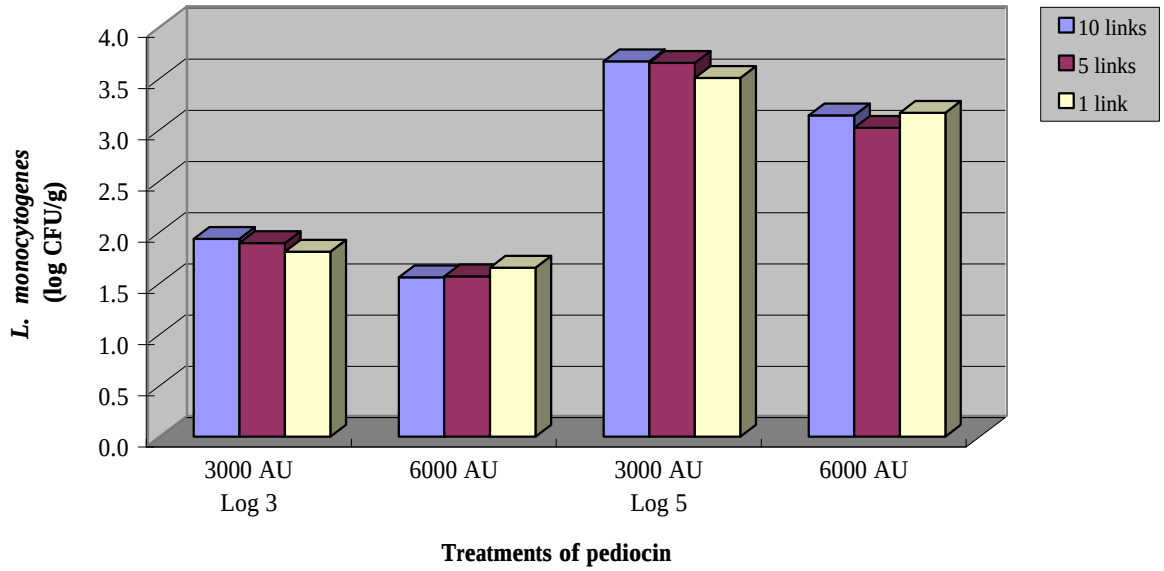
Results of this study showed that pediocin activity of the commercially available Alta 2341® significantly reduced the number of Lms on packaged frankfurters and delayed growth of the remaining cells during storage. The extent of the time delay was highly temperature-dependent. Post-packaging pasteurization by either thermal treatment or irradiation was very effective. Thermal treatment effectiveness was dependent on the package arrangement, temperature and time. Packages with frankfurter-to-frankfurter contact protected Lms cells from the heat process. Temperature of pasteurization had greater impact than time on surviving Lms in this study. No interaction or synergism occurred between pediocin treatments and thermal pasteurization. Irradiation, on the other hand, not only had a large impact on Lms, but also was synergistic with the pediocin treatments to affect Lms to an even greater extent when used in combination with pediocin.

Most of the quality characteristics of the frankfurters measured in this study were unaffected by the antilisterial treatments. The exceptions included color changes in lightness and redness. Products with pediocin and pasteurization treatments were darker and redder but the differences relative to controls were small. Sensory panel scores for texture resulted in a higher firmness score for the thermally-pasteurized frankfurters but this was not confirmed by the instrumental texture measurements.

Use of pediocin-treated casings or co-extrusion technology offers easy means to deliver bacteriocins or other compounds to the surface of frankfurters but further work is needed to improve antimicrobial effectiveness when these systems are used.

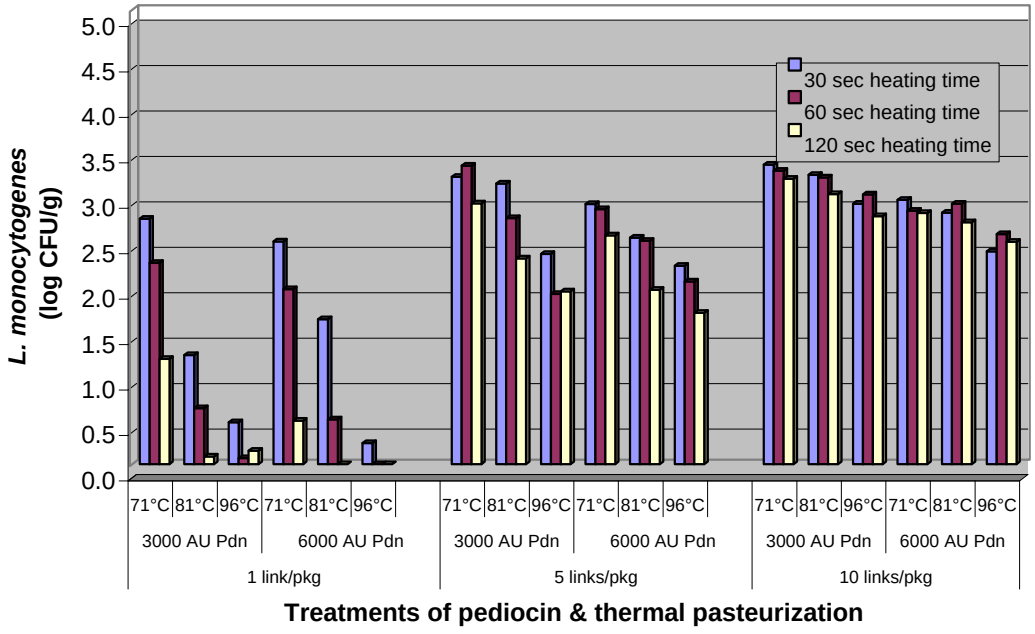
**Chart-01.AMI**

**Figure 1. Survival of *Listeria monocytogenes* (log 3 & 5) on the surface of frankfurters treated with pediocin in different package arrangements**



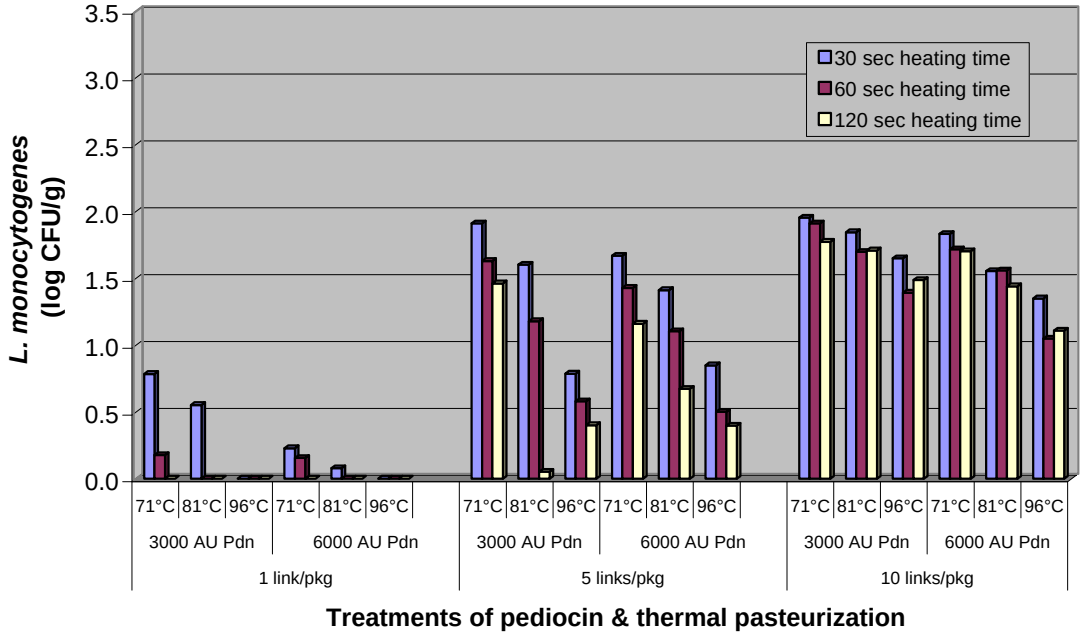

* 10 links: 10 links of frankfurters per package
* 5 links: 5 links of frankfurters per package
* 1 link: 1 link of frankfurter per package

**Figure 2. Survival of *Listeria monocytogenes* (log 5) on the surface of frankfurters treated with pediocin and post-packaging thermal pasteurization**




* Pdn: pediocin

**Figure 3. Survival of *Listeria monocytogenes* (log 3) on the surface of frankfurters treated with pediocin and post-packaging thermal pasteurization**




* Pdn: pediocin

**Figure 4. Survival of Listeria monocytogenes (log 3 & 5) on the surface of frankfurters treated with pediocin and irradiation**

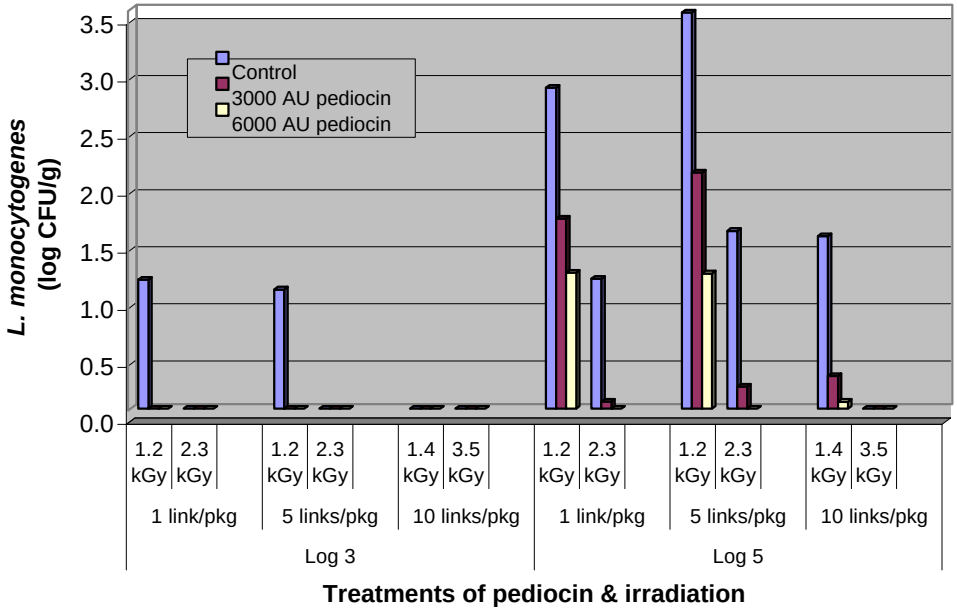
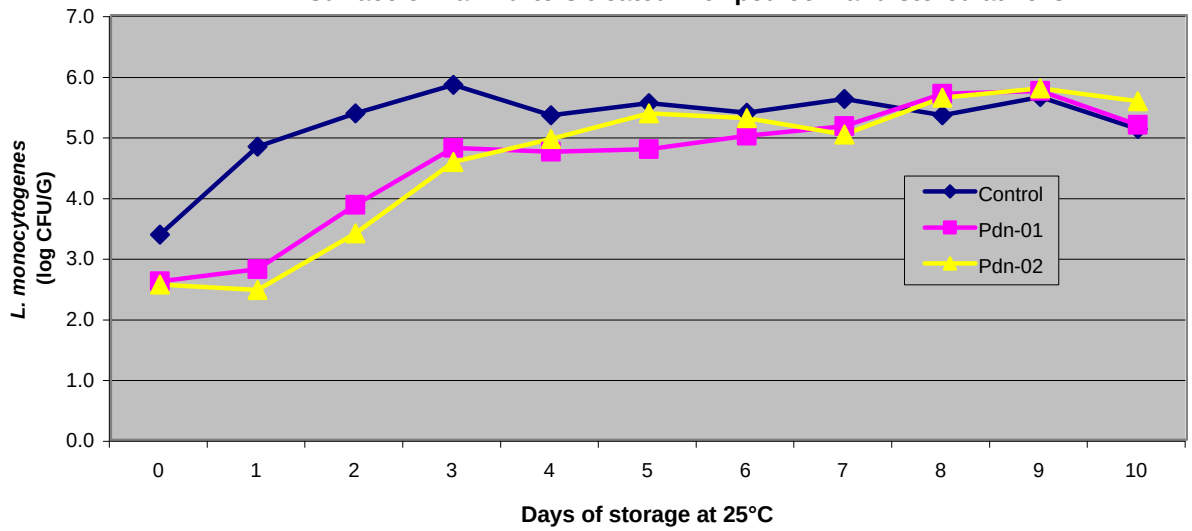
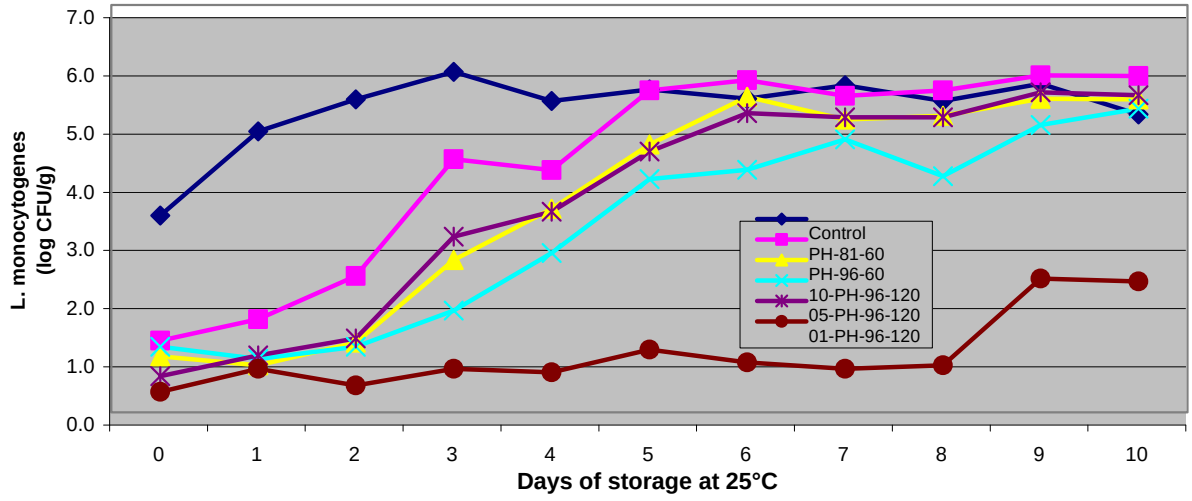


**Figure 5. Survival and growth of *Listeria monocytogenes* (log 3) on the surface of frankfurters treated with pediocin and stored at 25°C**



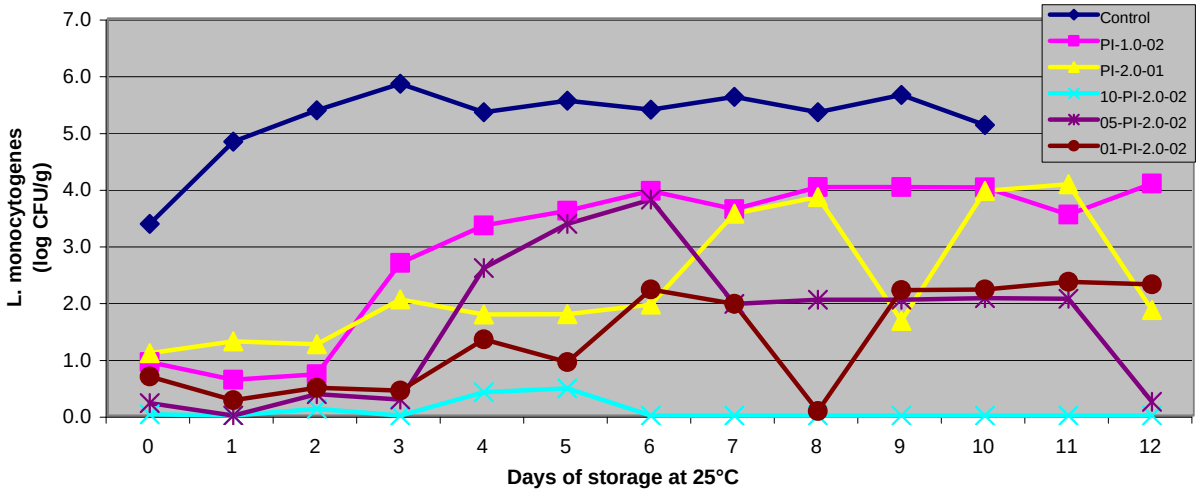

* Pdn-01: Frankfurters (5 links/pkg) treated with 3000 AU pediocin
* Pdn-02: Frankfurters (5 links/pkg) treated with 6000 AU pediocin

**Figure 6. Survival and growth of *Listeria monocytogenes* (log 3) on the surface of frankfurters treated with pediocin combined with post-packaging thermal pasteurization and stored at 25°C**



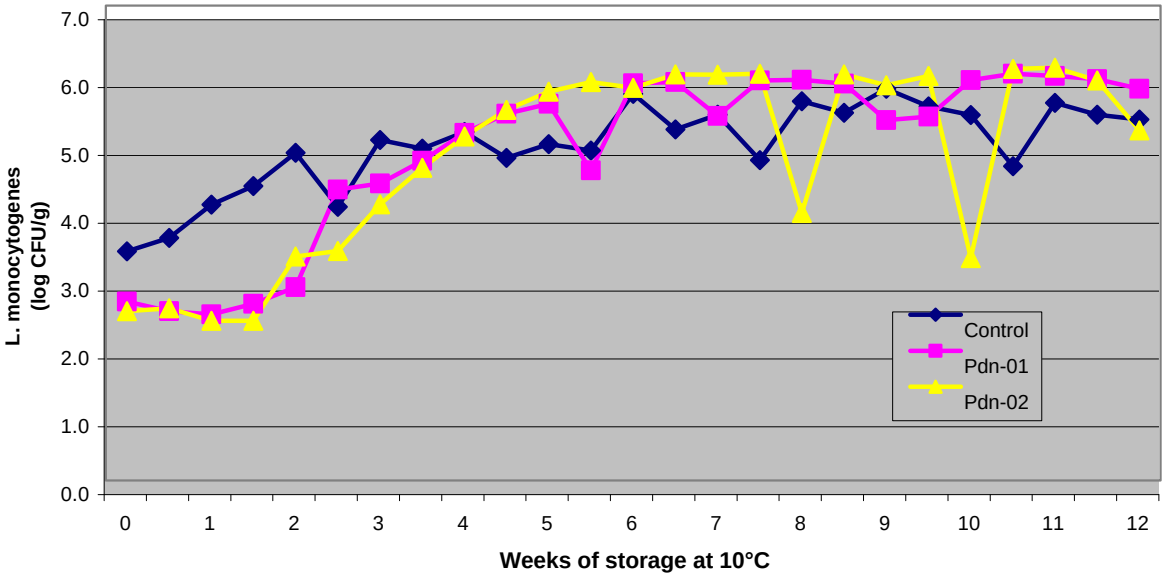

* PH-81-60: Frankfurters (5 links/pkg) treated with 6000 AU pediocin and heated in hot water at 81°C for 60 sec
* PH-96-60: Frankfurters (5 links/pkg) treated with 6000 AU pediocin and heated in hot water at 96°C for 60 sec
* 10-PH-96-120: Frankfurters (10 links/pkg) treated with 6000 AU pediocin and heated in hot water at 96°C for 120 sec
* 05-PH-96-120: Frankfurters (5 links/pkg) treated with 6000 AU pediocin and heated in hot water at 96°C for 120 sec
* 01-PH-96-120: Frankfurters (1 link/pkg) treated with 6000 AU pediocin and heated in hot water at 96°C for 120 sec

**Figure 7. Survival and growth of *Listeria monocytogenes* (log 3) on the surface of frankfurters treated with pediocin combined with irradiation and stored at 25°C**



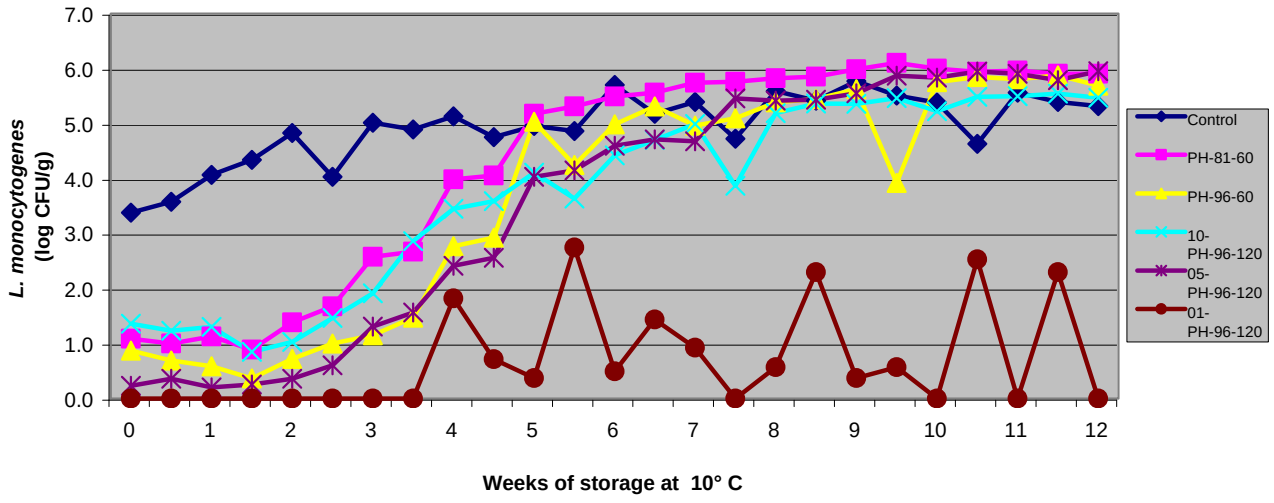

* PI -1.0-02: Frankfurters (5 links/pkg) treated with 6000 AU pediocin and irradiated with 1.2 kGy
* PI -2.0-01: Frankfurters (5 links/pkg) treated with 3000 AU pediocin and irradiated with 2.3 kGy
* 10-PI -2.0-02: Frankfurters (10 links/pkg) treated with 6000 AU pediocin and irradiated with 3.5 kGy
* 05-PI -2.0-02: Frankfurters (5 links/pkg) treated with 6000 AU pediocin and irradiated with 2.3 kGy
* 01-PI -2.0-02: Frankfurters (1 link/pkg) treated with 6000 AU pediocin and irradiated with 2.3 kGy

**Figure 8. Survival and growth of *Listeria monocytogenes* (log 3) on the surface of frankfurters treated with pediocin and stored at 10°C.**



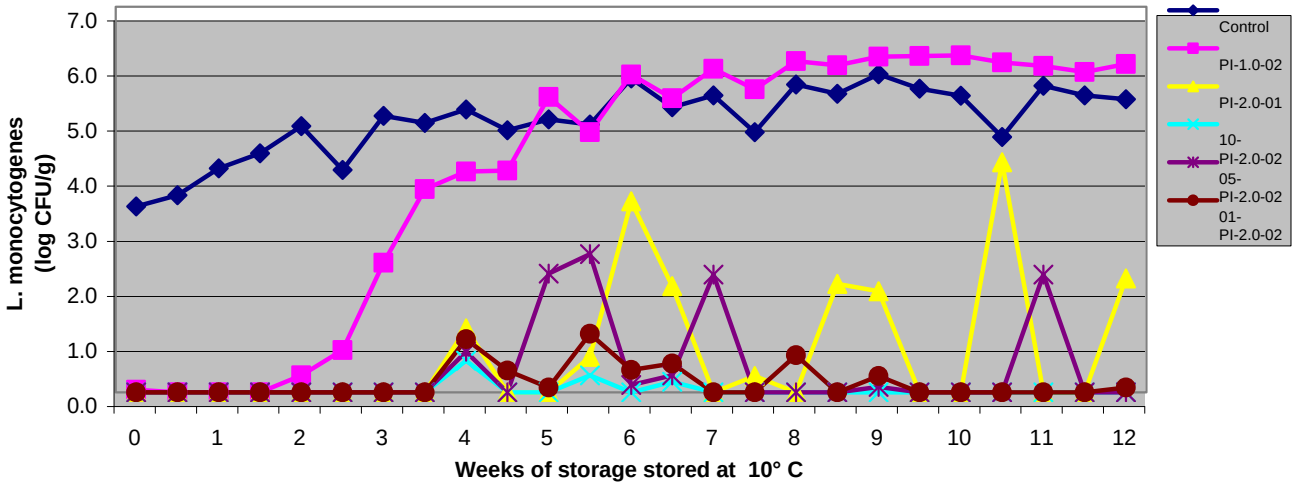

* Pdn-01: Frankfurters (5 links/pkg) treated with 3000 AU pediocin
* Pdn-02: Frankfurters (5 links/pkg) treated with 6000 AU pediocin

**Figure 9. Survival and growth of *Listeria monocytogenes* (log 3) on the surface of frankfurters treated with pediocin combined with post-packaging thermal pasteurization and stored at 10° C**



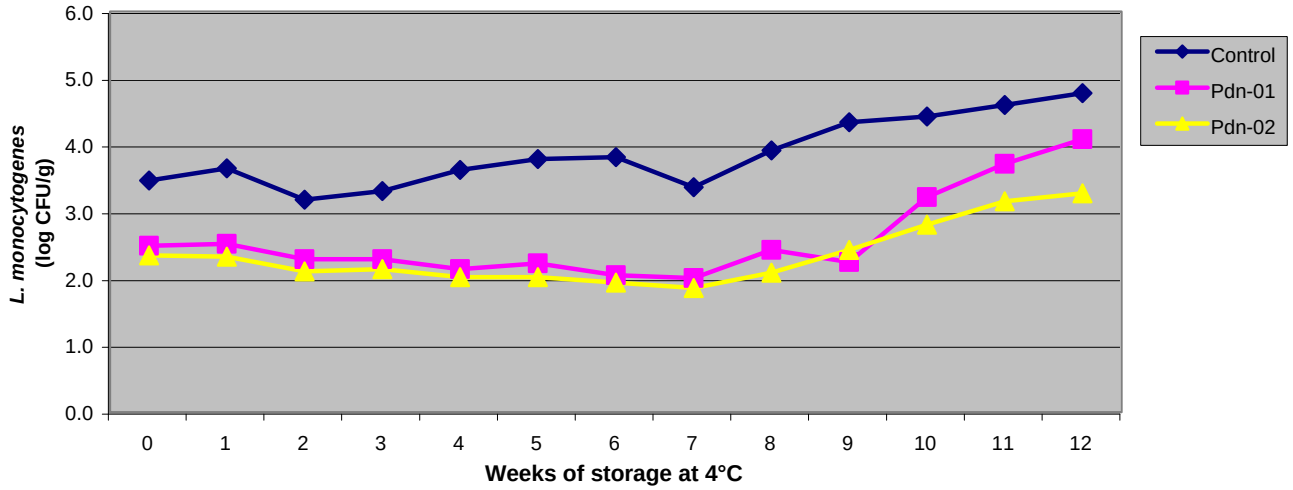

* PH-81-60: Frankfurters (5 links/pkg) treated with 6000 AU pediocin and heated in hot water at 81°C for 60 sec
* PH-96-60: Frankfurters (5 links/pkg) treated with 6000 AU pediocin and heated in hot water at 96°C for 60 sec
* 10-PH-96-120: Frankfurters (10 links/pkg) treated with 6000 AU pediocin and heated in hot water at 96°C for 120 sec
* 05-PH-96-120: Frankfurters (5 links/pkg) treated with 6000 AU pediocin and heated in hot water at 96°C for 120 sec
* 01-PH-96-120: Frankfurters (1 link/pkg) treated with 6000 AU pediocin and heated in hot water at 96°C for 120 sec

**Figure 10. Survival and growth of *Listeria monocytogenes* (log 3) on the surface of frankfurters treated with pediocin combined with irradiation and stored at 10° C**



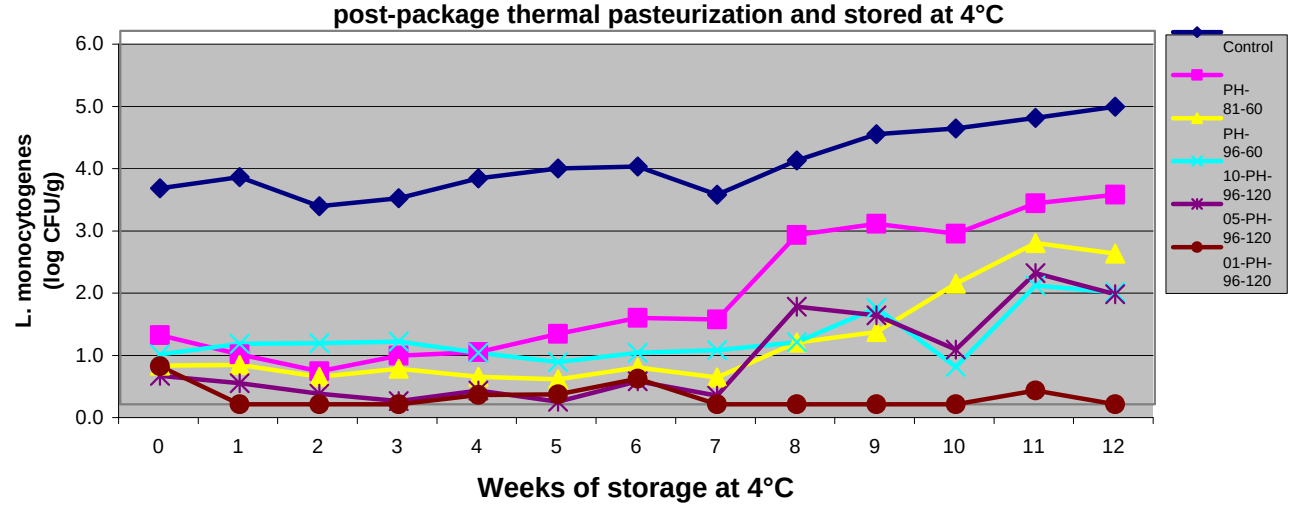

* PI -1.0-02: Frankfurters (5 links/pkg) treated with 6000 AU pediocin and irradiated with 1.2 kGy
* PI -2.0-01: Frankfurters (5 links/pkg) treated with 3000 AU pediocin and irradiated with 2.3 kGy
* 10-PI -2.0-02: Frankfurters (10 links/pkg) treated with 6000 AU pediocin and irradiated with 3.5 kGy
* 05-PI -2.0-02: Frankfurters (5 links/pkg) treated with 6000 AU pediocin and irradiated with 2.3 kGy
* 01-PI -2.0-02: Frankfurters (1 link/pkg) treated with 6000 AU pediocin and irradiated with 2.3 kGy

**Figure 11. Survival and growth of *Listeria monocytogenes* (log 3) on the surface of frankfurters treated with pediocin and stored at 4°C**



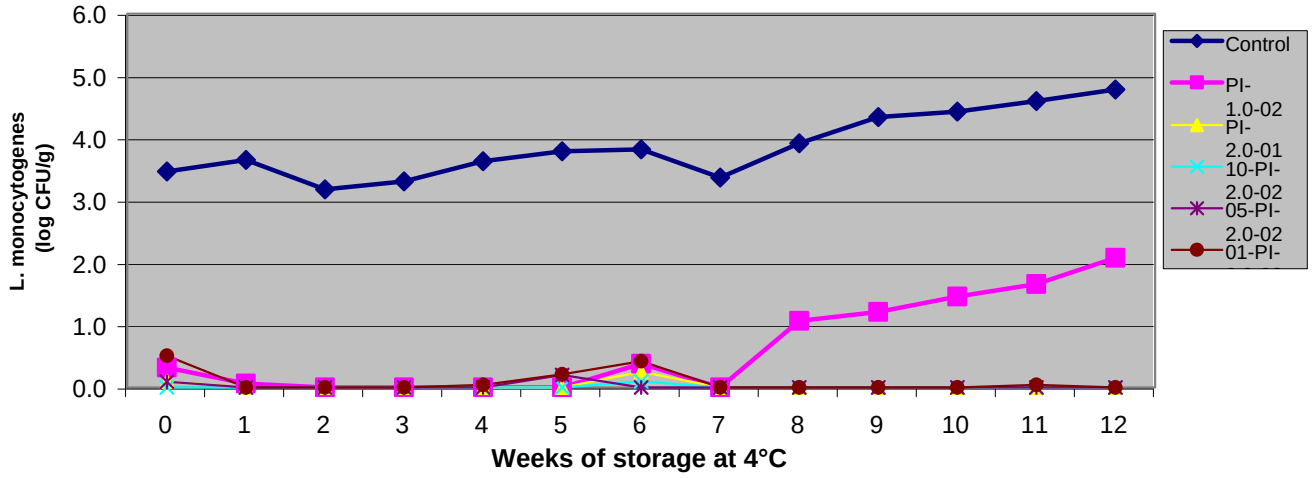

* Pdn-01: Frankfurters (5 links/pkg) treated with 3000 AU pediocin
* Pdn-02: Frankfurters (5 links/pkg) treated with 6000 AU pediocin

**Figure 12. Survival and growth of *Listeria monocytogenes* (log 3) on the surface of frankfurters treated with pediocin combined with post-package thermal pasteurization and stored at 4°C**



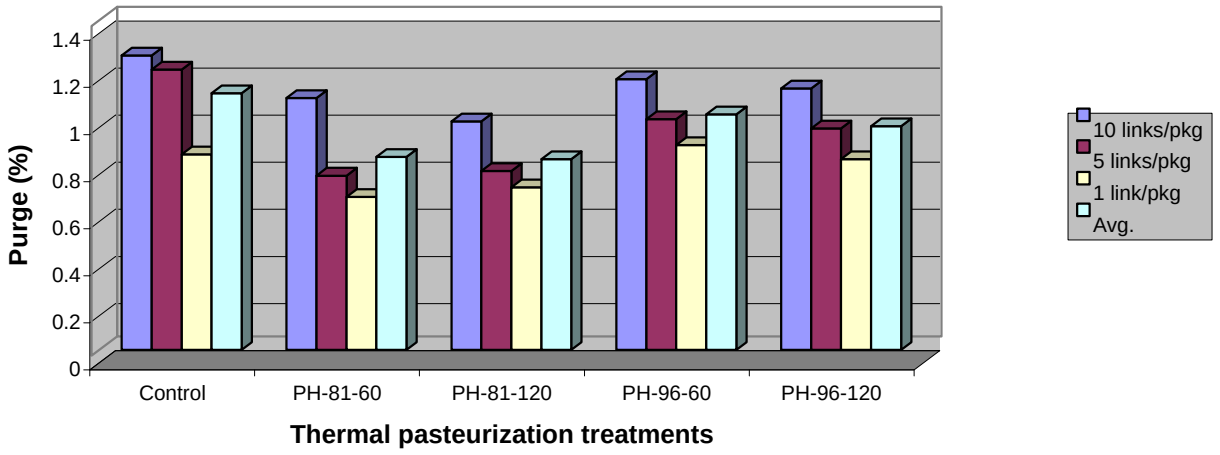

* PH-81-60: Frankfurters (5 links/pkg) treated with 6000 AU pediocin and heated in hot water at 81°C for 60 sec
* PH-96-60: Frankfurters (5 links/pkg) treated with 6000 AU pediocin and heated in hot water at 96°C for 60 sec
* 10-PH-96-120: Frankfurters (10 links/pkg) treated with 6000 AU pediocin and heated in hot water at 96°C for 120 sec
* 05-PH-96-120: Frankfurters (5 links/pkg) treated with 6000 AU pediocin and heated in hot water at 96°C for 120 sec
* 01-PH-96-120: Frankfurters (1 link/pkg) treated with 6000 AU pediocin and heated in hot water at 96°C for 120 sec

**Figure 13. Survival and growth of *Listeria monocytogenes* (log 3) on the surface of frankfurters treated with pediocin combined with irradiation and stored at 4°C**



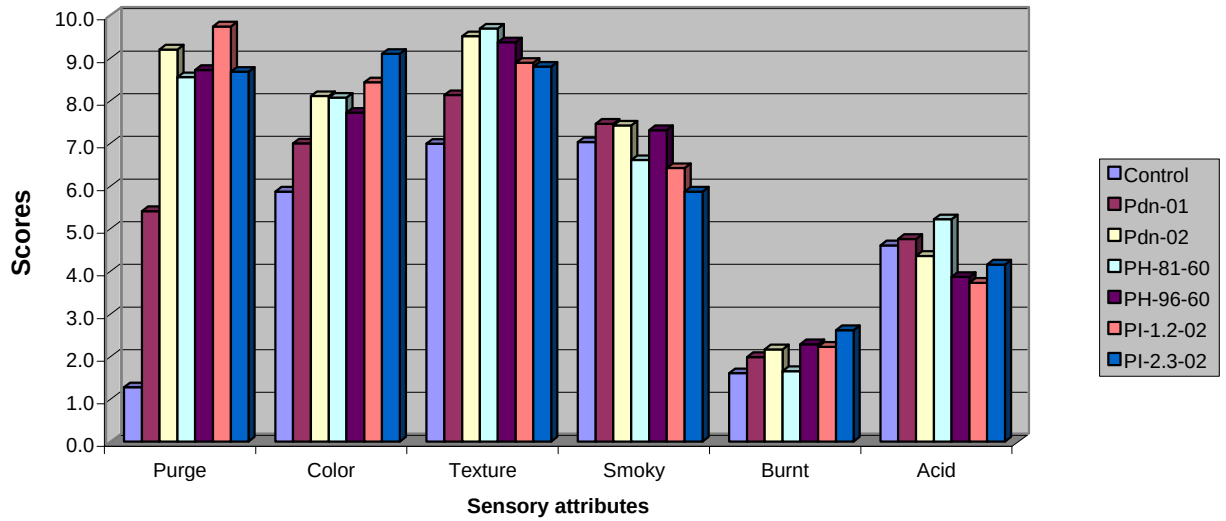

* PI -1.0-02: Frankfurters (5 links/pkg) treated with 6000 AU pediocin and irradiated with 1.2 kGy
* PI -2.0-01: Frankfurters (5 links/pkg) treated with 3000 AU pediocin and irradiated with 2.3 kGy
* 10-PI -2.0-02: Frankfurters (10 links/pkg) treated with 6000 AU pediocin and irradiated with 3.5 kGy
* 05-PI -2.0-02: Frankfurters (5 links/pkg) treated with 6000 AU pediocin and irradiated with 2.3 kGy
* 01-PI -2.0-02: Frankfurters (1 link/pkg) treated with 6000 AU pediocin and irradiated with 2.3 kGy

**Figure 14. Purge accumulation (%) of frankfurters after post-package thermal pasteurization**




* PH-81-60: Frankfurters [10, 5 or 1 link(s)/pkg] heated in hot water at 81°C for 60 sec after packed
* PH-81-120: Frankfurters [10, 5 or 1 link(s)/pkg] heated in hot water at 81°C for 120 sec after packed
* PH-96-60: Frankfurters [10, 5 or 1 link(s)/pkg] heated in hot water at 96°C for 60 sec after packed
* PH-96-120: Frankfurters [10, 5 or 1 link(s)/pkg] heated in hot water at 96°C for 120 sec after packed

**Figure 15. Sensory evaluation of frankfurters**




* Pdn-01: Frankfurters (5 links/pkg) treated with 3000 AU pediocin
* Pdn-02: Frankfurters (5 links/pkg) treated with 6000 AU pediocin
* PH-81-60: Frankfurters (5 links/pkg) treated with 6000 AU pediocin and heated in hot water at 81°C for 60 sec
* PH-96-60: Frankfurters (5 links/pkg) treated with 6000 AU pediocin and heated in hot water at 96°C for 60 sec
* PI-1.2-02: Frankfurters (5 links/pkg) treated with 6000 AU pediocin and irradiated with 1.2 kGy
* PI-2.3-02: Frankfurters (5 links/pkg) treated with 6000 AU pediocin and irradiated with 2.3 kGy

Table-01.AMI

**Table 1. Comparisons of analyses of frankfurters**

| | Purge (%) | Color L | Color a | Color b | Firmness (g) Skin | Firmness (g) Interior | pH | TBA value (mg MDA/kg) |
|---|---|---|---|---|---|---|---|---|
| **Control** | 0.61 | 45.29 | 15.21 | 13.44 | 0.229 | 0.106 | 6.12 | 0.64 |
| | ( a [7]) | ( a ) | ( a ) | ( a ) | ( a ) | ( a ) | ( a ) | ( a ) |
| **Pdn-01** [1] | 3.33 | 44.26 | 15.92 | 13.33 | 0.220 | 0.110 | 6.08 | 0.67 |
| | ( c ) | ( ac ) | ( ac ) | ( a ) | ( a ) | ( a ) | ( b ) | ( a ) |
| **Pdn-02** [2] | 5.13 | 42.78 | 16.04 | 13.34 | 0.222 | 0.113 | 6.05 | 0.70 |
| | ( b ) | ( bc ) | ( bc ) | ( a ) | ( a ) | ( a ) | ( b ) | ( a ) |
| **PH-81-60** [3] | 5.76 | 42.33 | 16.34 | 13.27 | 0.233 | 0.116 | 6.05 | 0.52 |
| | ( b ) | ( b ) | ( b ) | ( a ) | ( a ) | ( a ) | ( b ) | ( a ) |
| **PH-96-60** [4] | 5.35 | 42.21 | 16.56 | 13.48 | 0.292 | 0.181 | 6.02 | 0.78 |
| | ( b ) | ( b ) | ( b ) | ( a ) | ( a ) | ( a ) | ( ab ) | ( a ) |
| **PI-1.2-02** [5] | 5.38 | 42.3 | 16.34 | 13.29 | 0.254 | 0.121 | 6.03 | 0.72 |
| | ( b ) | ( b ) | ( b ) | ( a ) | ( a ) | ( a ) | ( b ) | ( a ) |
| **PI-2.3-02** [6] | 6.08 | 42.17 | 16.22 | 13.49 | 0.243 | 0.113 | 6.04 | 0.70 |
| | ( b ) | ( b ) | ( b ) | ( a ) | ( a ) | ( a ) | ( b ) | ( a ) |

1. Pdn-01: Frankfurters (5 links/pkg) treated with 3000 AU pediocin
2. Pdn-02: Frankfurters (5 links/pkg) treated with 3000 AU pediocin
3. PH-81-60: Frankfurters (5 links/pkg) treated with 6000 AU pediocin and heated in hot water at 81°C for 60 sec
4. PH-96-60: Frankfurters (5 links/pkg) treated with 6000 AU pediocin and heated in hot water at 96°C for 60 sec
5. PI-1.2-02: Frankfurters (5 links/pkg) treated with 6000 AU pediocin and irradiated with 1.2 kGy
6. PI-2.3-02: Frankfurters (5 links/pkg) treated with 6000 AU pediocin and irradiated with 2.3 kGy
7. a, b, c means in the same column with the same letter are not different (p > 0.05)

**Table 2.Comparisons of delivery systems for inhibitors**

| Agars [1] | *Listeria monocytogenes* (log CFU/g) Normal casing Control | Normal casing Pdn [4] | Special casing [2] Control | Special casing [2] Pdn + D.A. | Co-extrusion [3] Control | Co-extrusion [3] Pdn |
|---|---|---|---|---|---|---|
| **Mox** | 3.53 | 3.02 | 3.37 | 3.31 | 3.61 | 3.47 |
| | (ac [5]) | (b) | (ac) | (c) | (a) | (ac) |
| **TSA-YE** | 3.65 | 3.15 | 3.32 | 3.43 | 3.58 | 3.48 |
| | (a) | (b) | (ab) | (ab) | (a) | (ab) |

1. Agars for *Listeria monocytogenes* recovery
2. Casing interior coating of pediocin or pediocin and sodium diacetate
3. Townsend Kontura system
4. Pediocin-treated
5. a, b, c means in the same column with the same letter are not different (p > 0.05)