

American Meat Institute Research Foundation  
Final Report

Pre- and Post-package Pasteurization of RTE Meats for Reduction of  
*Listeria monocytogenes*

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A. Pre-package pasteurization of hotdogs

1. To use a “hotdog pasteurizer” to determine the effectiveness of a hot water deluge system for pasteurization of hotdogs.

The Unitherm Hotdog Pasteurizer is a convenient self-contained apparatus for hotdog pasteurization, requiring water and electrical connections. The self-contained heating unit can be used to ‘dial-in’ any temperature from ambient to high temperatures (205°F) allowing up to 2.25-log reduction for a 10-sec rinse (Fig. 1). One drawback is that the hotdogs are readily heated internally (Fig. 2) and representatives of several hotdog manufacturers have indicated that this would have to be alleviated for the process to have commercial applicability. The manufacturer was to have provided a contiguous chiller for the system to assess thermal lethality in conjunction with an automated chiller, but this was not provided as originally planned.

The “Hotdog pasteurizer” described above was examined at 165°, 180°F, and 195°F for 15, 20, and 25-sec dwell times (Fig. 3). The data shows that the shortest dwell time used (15-sec) at 165°F gave a significant reduction beyond what was rinsed off with chilled water and this may be considered a significant post-process lethality step against *Listeria*. Greater reductions were observed with 180°F but further reductions at 195°F did not show a significant difference over that observed at 180°F. The company did not provide the promised ‘chiller

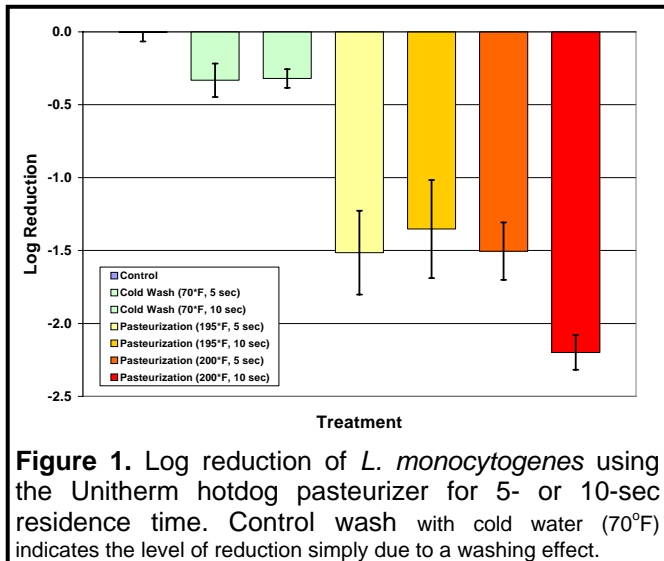


Figure 1. Log reduction of *L. monocytogenes* using the Unitherm hotdog pasteurizer for 5- or 10-sec residence time. Control wash with cold water (70°F) indicates the level of reduction simply due to a washing effect.

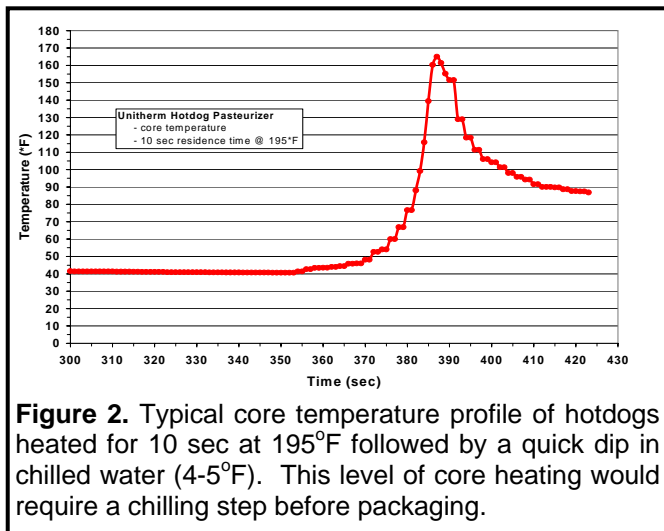
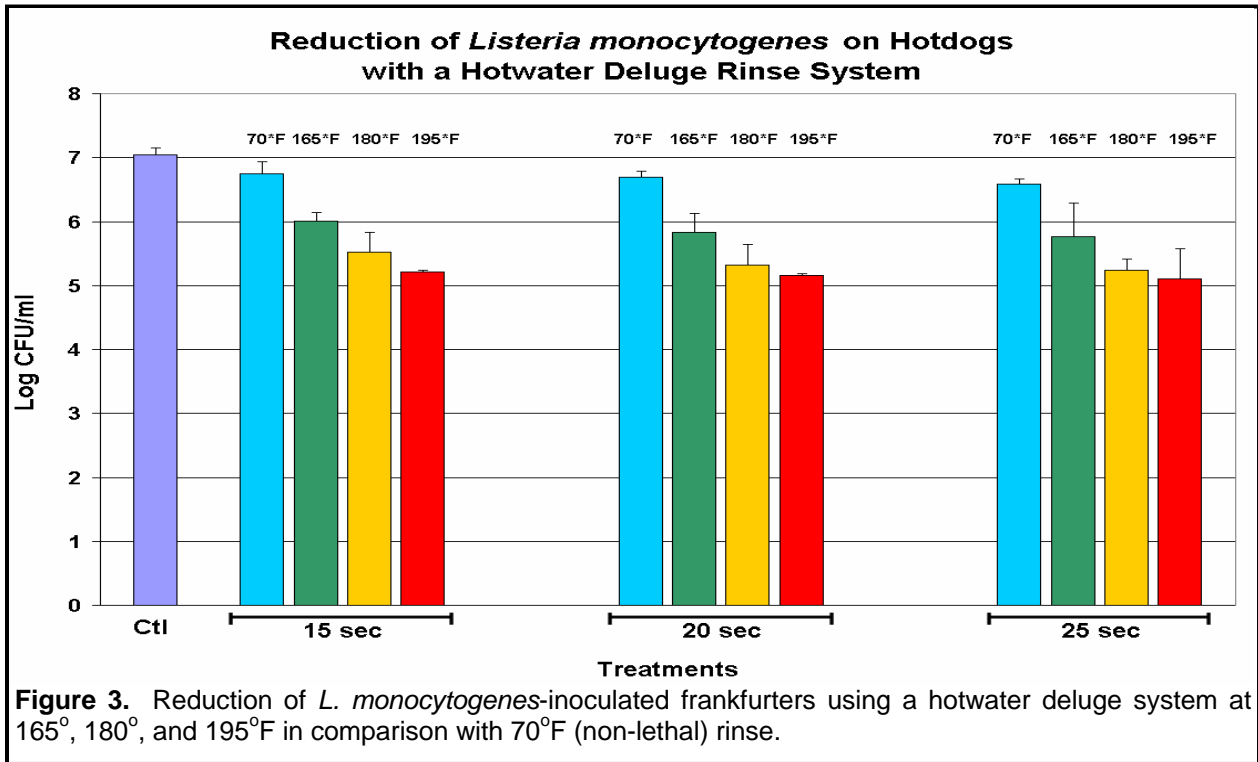


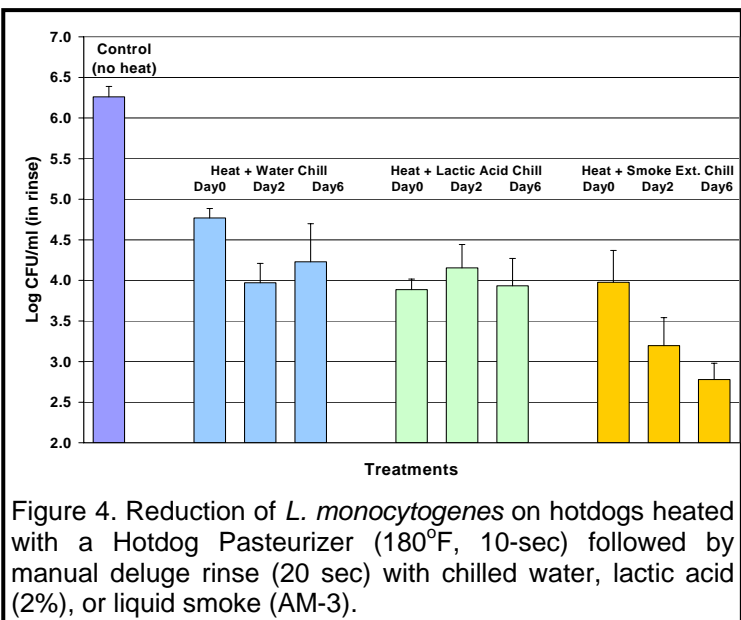
Figure 2. Typical core temperature profile of hotdogs heated for 10 sec at 195°F followed by a quick dip in chilled water (4-5°F). This level of core heating would require a chilling step before packaging.

system' to accompany its pasteurizer that would have involved further testing since the short but high-temperature heating increased internal temperatures such that they should be chilled (Fig. 2) before packaging. However, the hotdog pasteurizer is a convenient method to apply liquid antimicrobials in a controlled manner that could accommodate heating of the antimicrobial (not necessarily to high temperatures) and which would facilitate recirculation.



2. **To use antimicrobials, and/or organic acid sprays, in combination with pre- and/or post-package pasteurization for reduction of *L. monocytogenes* to determine if equivalent reductions can be obtained with less processing time or temperatures.**

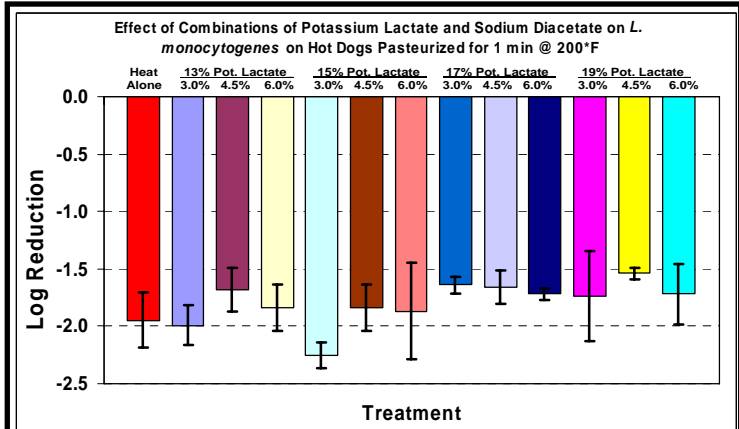
**Lactate and diacetate.** We have examined the hotdog pasteurizer in conjunction with manual chilled rinse, using a 10-sec heating at 180°F followed by a 20 sec rinse using either chilled (~41°F) water, lactic acid (2%) or liquid smoke (AM-3) in which plate counts were examined at 0 time (immediately after treatment), and after 2- and 6-days storage at 35°F. Both lactic acid and liquid smoke rinses gave approximately 0.75-1 log greater reductions immediately after heat treatment than obtained with simple chilled water rinsing. However, levels of *L. monocytogenes* continued to decline by day 2 and again by day



6 which did not occur with the other rinse solutions, suggesting that a chilled antimicrobial rinse could have implications to extend microbial reduction during shelf-life. The Unitherm hotdog pasteurizer may also provide an easy means to provide an antimicrobial deluge even without heating as the flow is captured in the underlying reservoir which can be subsequently heated to eliminate any viable cells that have washed off the inoculated samples (or the cells killed by the antimicrobial itself).

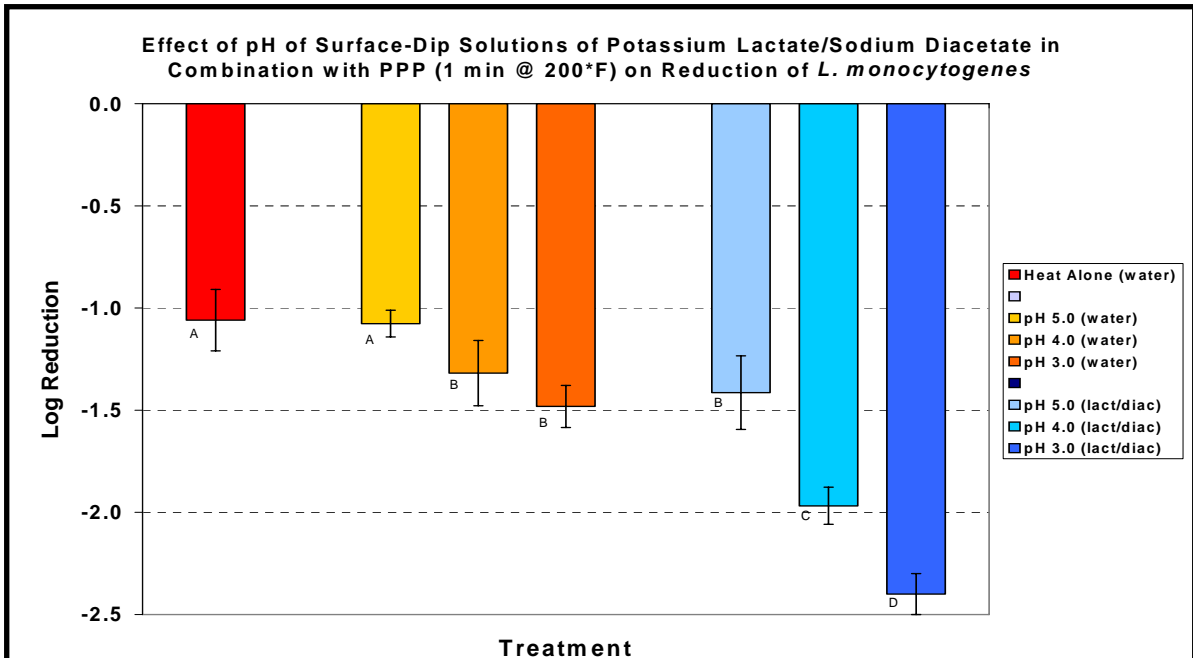
In a prior study (unrelated to this project), dip treatments of hotdogs in various combinations of lactate/diacetate had little or no effect on reduction of *L. monocytogenes*-inoculated hotdogs compared to heat treatment alone (Fig. 5). We had presumed that possibly pH adjustment may allow a greater proportion of undissociated molecules (higher [H<sup>+</sup>]-ion concentration at lower pH levels) whereby more molecules may be transported into microbial cells where they would disassociate in the cell because the cytoplasmic pH is above the pK<sub>a</sub> of the acids.

This was performed by comparing hotdogs dipped in sterile water (no pH adjustment) with hotdogs dipped in water adjusted to pH 5, pH 4, and pH 3 (with HCl), and to hotdogs dipped in a sodium diacetate(4.5%)/potassium lactate (17%) mixture adjusted to pH 5, pH 4, and pH 3 (Fig. 6). The data shows that, under the heating conditions used, heat alone (control) resulted in ~1.1-log reduction of *L. monocytogenes* when pasteurized. Similarly heated products that were dipped in water adjusted to pH 5, 4, and 3, although showing slightly increasing trends in



**Figure 5.** Surface treatment (dip) of hotdogs in various combinations of lactate/diacetate prior to pasteurization (1 min @ 200°F).

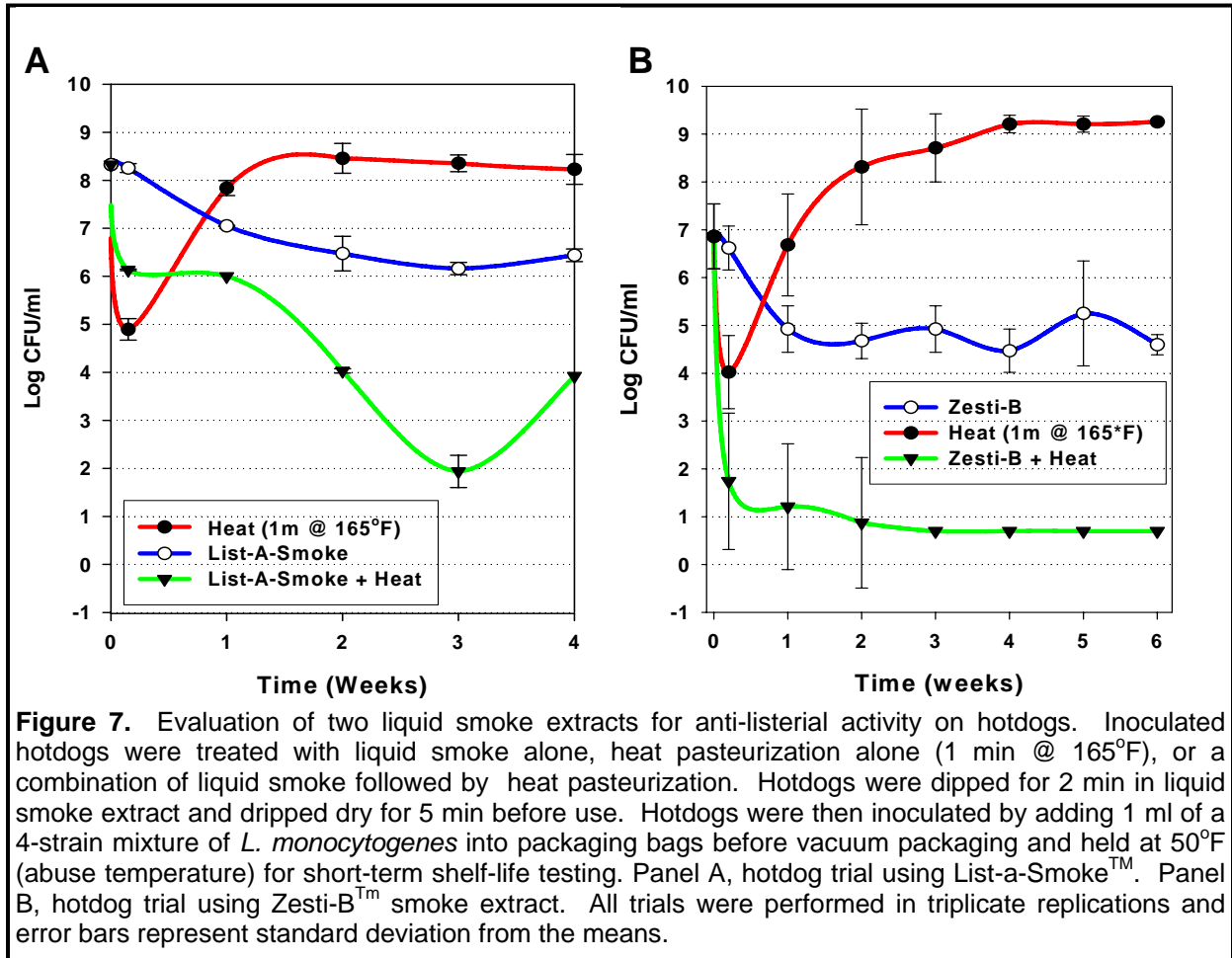
with hotdogs dipped in water adjusted to pH 5, pH 4, and pH 3 (with HCl), and to hotdogs dipped in a sodium diacetate(4.5%)/potassium lactate (17%) mixture adjusted to pH 5, pH 4, and pH 3 (Fig. 6). The data shows that, under the heating conditions used, heat alone (control) resulted in ~1.1-log reduction of *L. monocytogenes* when pasteurized. Similarly heated products that were dipped in water adjusted to pH 5, 4, and 3, although showing slightly increasing trends in



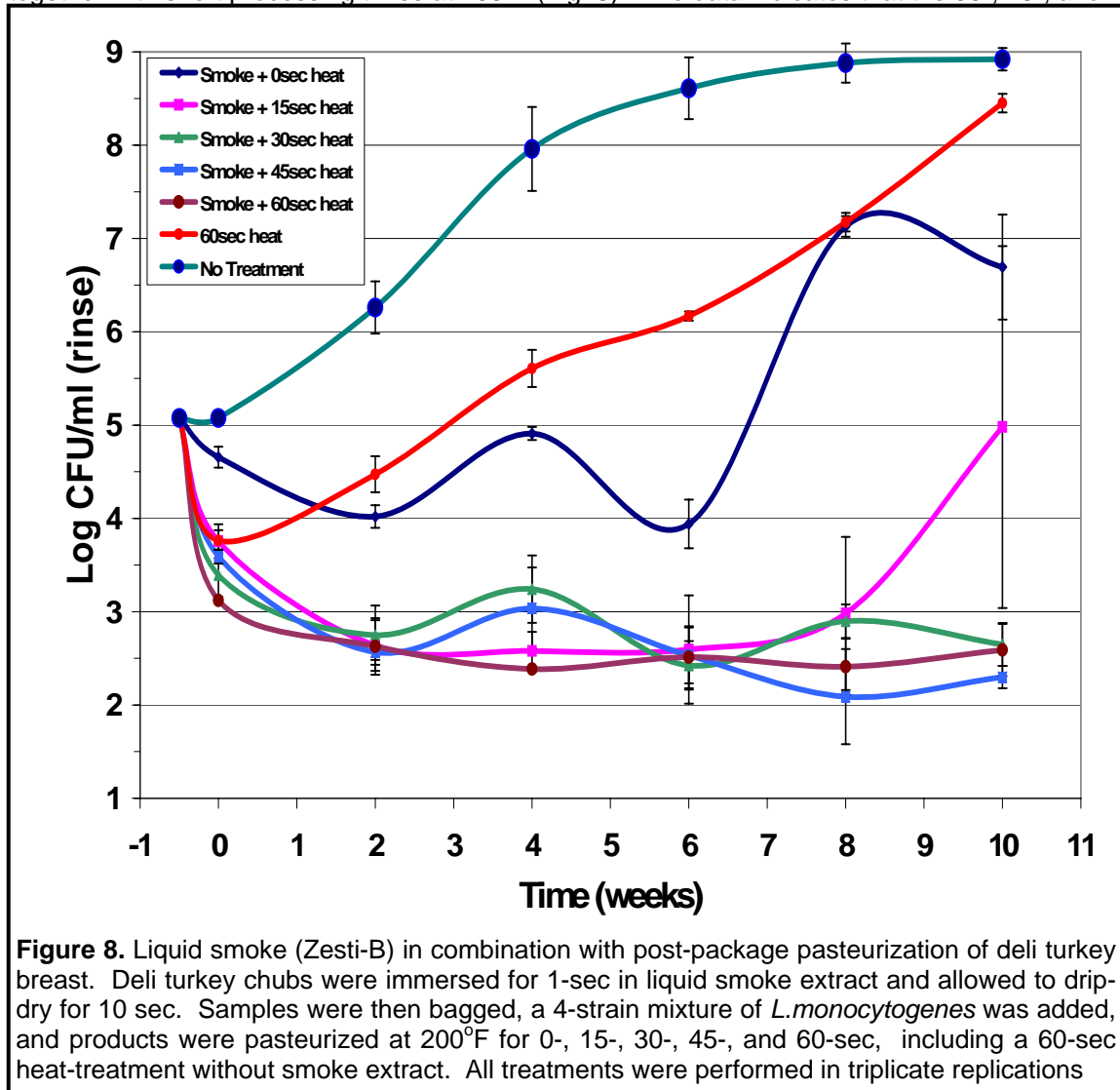
**Figure 6.** The effect of pH of a lactate/diacetate solution vs. pH-adjusted water in dip treatments on reduction of *L. monocytogenes* during heat pasteurization. Data bars with the same letter designation are not significantly different ( $p > 0.05$ )

reduction were only slightly greater at pH 3.0 showing a reduction of ~1.45-logs. However, with sodium diacetate/potassium lactate solutions adjusted to the same pH levels, the hotdogs showed significant differences with greater reductions in *Listeria*, not only from the control, but from each other as well (Fig. 6; 1.4-2.4 log reduction attributed to pH vs. 1.06 log reduction by heat alone). It is not clear what impact this could have beyond initial reductions as pH at the surface may readily be buffered by dilution/diffusion with time, but at least may explain why our previous study did not show much impact when using even slightly acidified pH (~5.0-5.5) levels. However, this may lend further credence towards use of lactate/diacetate pH-adjusted dips whereby prior research has shown that surface lactate/diacetate treatments are not effective against *Listeria*.

**Liquid smoke condensates.** Liquid smoke extracts (List-a-Smoke and Zesti-B) were examined in conjunction with post-package pasteurization using hotdogs as a test meat matrix for the combination of liquid smoke condensates and heat (Fig. 7). The data shows that heat alone gives an initial reduction of *L. monocytogenes*, whereby residual cells, if unprevented, could grow out to higher levels. Smoke alone shows a slow and modest reduction that holds through the abuse temperature conditions of the shelf life (Fig. 7). However, liquid smoke plus heat (especially Zesti-B) showed an impressive reduction that was maintained throughout the short shelf life period. In prior work with post-package pasteurization alone, we have been able to achieve a 2-4 log reduction of *L. monocytogenes* with submersed water post-package pasteurization at 200°F, however, the extended processing times of 2-4 min, has often produced excessive purge that is not aesthetically pleasing to clients nor helpful to shelflife. We examined a 1-sec immersion in liquid smoke extract that has been shown to be effective (see below)

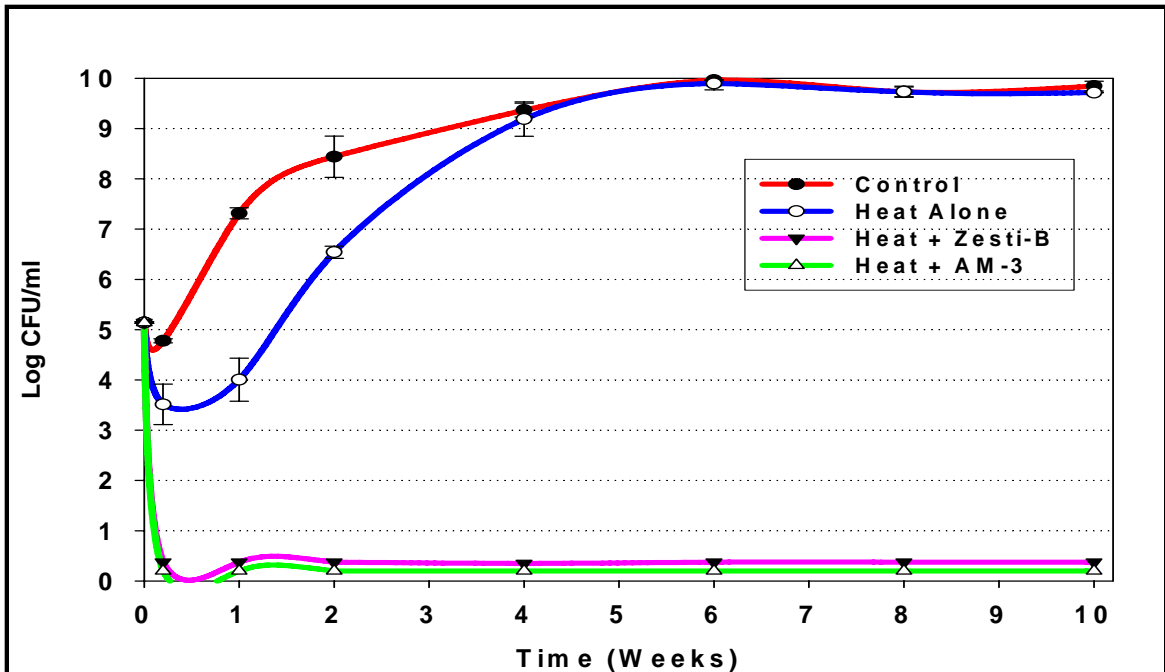


together with short processing times at 200°F (Fig. 8). The data indicates that the 30-, 45-, and



60-sec treatments, in combination with the liquid smoke extract were capable of initially reducing *L. monocytogenes* to levels lower than the heat process alone and prevented the emergence of residual *Listeria* (Fig. 8). This should be of greater significance when considering practical levels (i.e., lower) of *L. monocytogenes* that might be acquired if in-house contamination were to occur. However, the use of low, practical inoculum levels has the disadvantage of not seeing the trend of inhibition that can be observed by using higher levels.

One processor we've worked with indicated that Zesti-B had too much color and flavor for their product line, so we compared Zesti-B with AM-3, an extract that is colorless and has much reduced flavor than the Zesti-B product. AM-3 was developed for use in a greater range of products than just those that would require a 'smoke flavor'. This experiment was done in conjunction with heating (1 min @ 165°F), and included non-treated, non-heated control, a heat-only control (no smoke), and Zesti-B or AM-3-treated hotdogs (1 sec dip) heated for 1 min at 165°F (Fig. 9). All hotdogs were inoculated with ~10<sup>5</sup> CFU into the bag before vacuum packaging and held at 43°F. As you can see from Fig. 9, the *L. monocytogenes* inoculum starts increasing almost immediately in the control. The heat-only control gives a modest 1.5-log decrease, whereas heat+smoke, whether Zesti-B or AM-3 gives no detectable *Listeria*

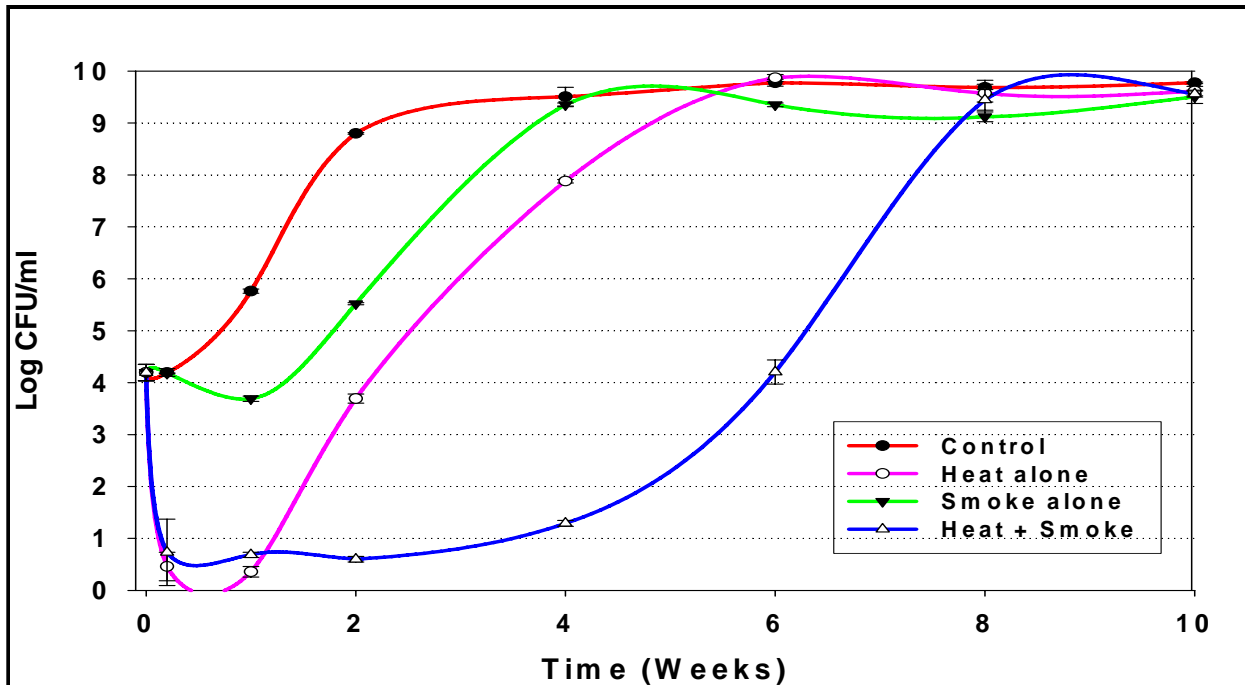


**Figure 9.** Effect of Zesti-B™ (brown) vs. AM-3™ (clear) liquid smoke in combination with heat against *Listeria monocytogenes* on hotdogs. Hotdogs were dipped in either liquid smoke extract for 1 sec, allowed to drip dry for 10 sec, and inoculated by adding 1-ml of a 4-strain mixture of *L. monocytogenes* into packaging bags before vacuum packaging. Treatments were no heat/no smoke (control), heat treatment alone, and liquid smoke treatment plus heat. Heat pasteurization was accommodated by post-package pasteurization in hot water for 1 min at 73.9°C (165°F). Samples were held at 50°F (abuse temperature) for shelf-life testing and plated at 1 hr, 1 wk, 2 wk, and biweekly thereafter for up to 10 weeks.

immediately after treatment or thereafter through 10 weeks of shelflife, confirming what we have seen with post-package pasteurization studies that the inhibitory effect of liquid smoke is

enhanced by heating. The results indicate that Zesti-B and AM-3 had similar inhibitory properties and AM-3 could be used where less color was desired.

Our previous results using liquid smoke in combination with *post-package* (in-bag) pasteurization showed that reduced time of heating (below those typically used in postpackage pasteurization) could be used to reduce and suppress outgrowth of *L. monocytogenes* for up to 10 weeks. In the current series of trials, we examined *pre-package* pasteurization using a radiant heat oven on prepped (1-sec) deli turkey chubs manufactured without lactate/diacetate and processed for 60-sec for which we would normally could obtain a 2-3.5 log reduction, depending on the product (Fig. 10). Pre-package and post-package pasteurization are post-process lethality steps that can be used to obtain Alternative 2 process category. Our results showed that inoculated but untreated controls grew readily while the unheated smoke-treated samples observed a slight reduction before allowing outgrowth to occur. With heat-treated samples, heat alone resulted in near-undetectable levels immediately after processing with outgrowth occurring by week 2 whereby smoke-plus-heat treatment showed similar initial reductions and suppression of outgrowth for up to 4 weeks before outgrowth occurred. It is possible that the higher surface temperatures obtained by radiant heating may 'burn off' or affect the residual liquid smoke on the surface, reducing its potential effect compared to what we have observed with submerged water in-bag pasteurization. Also, the turkey products we've tested to not behave the way hotdog-type products react when in regard to the combination of liquid smoke plus heat (i.e., hotdogs show a more effective heat+smoke reaction during shelf life). Overall, our results with liquid smoke applications on deli turkey (smoke alone or in combination with heat) are not as impressive as



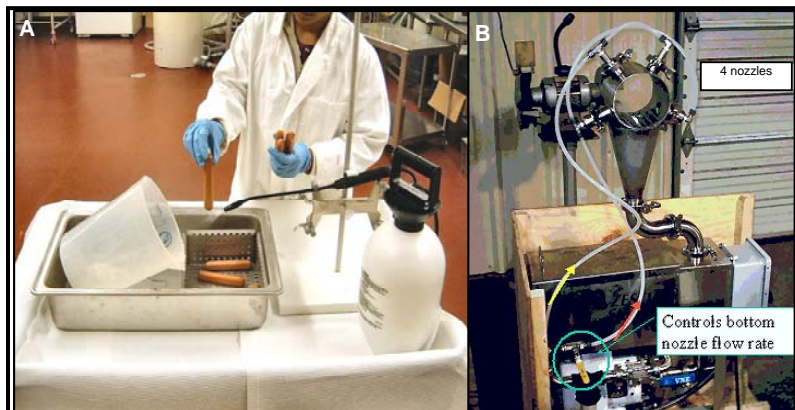
**Figure 10.** Liquid smoke (Zesti-BT<sup>m</sup>) in combination with pre-package pasteurization of 2-4 lb deli turkey breasts manufactured without lactate/diacetate. Deli turkey chubs were immersed for 1-sec in liquid smoke extract, allowed to drip-dry for 10 sec, and 1-ml of a 4-strain mixture of *L. monocytogenes* was dribbled over the surface and spread with a gloved-finger. The products were then heated for 60-sec in a radiant heat oven (~550oF air temperature) used for pre-package pasteurization of RTE deli meats. All treatments were performed in triplicate replications and error bars represent standard deviation from the mean.

what we have observed with hotdogs (see below), and may be due to either the size dimension of the nature of the product tested in regard to the product formulation and liquid smoke interaction.

### 3. To determine the impact of such antimicrobials/organic acids on outgrowth of *Listeria monocytogenes* during shelf life.

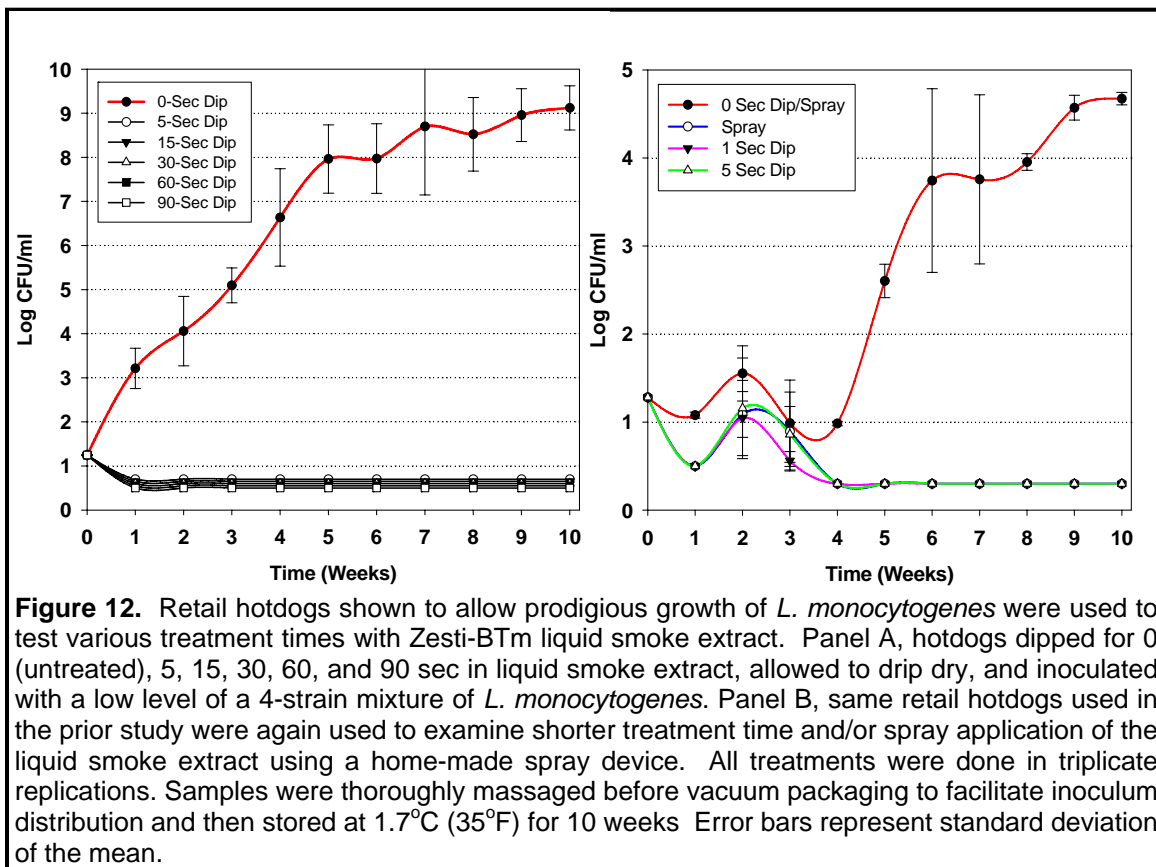
**Liquid smoke extracts.** We initially tested liquid smoke condensates as part of heating experiments whereby one of the controls was a 'smoke alone' treatment. In further examining the effect of liquid smoke condensates, we tested various dip and spray treatments.

For spray treatments, we used in-house pressurized canisters and commercial supplied spray equipment (Fig. 11).



**Figure 11.** Spray applicators used in this study. Panel A, home-made device for spraying liquid smoke extracts onto samples in our laboratory. Hotdogs were dropped through the spray mist after locking the handle in the spray position. Panel B, commercial device with 4 nozzles and pump mechanism to re-circulate unused solution. The device is attached to the outside of a peeler unit and hotdogs are sprayed as they eject through the opening.

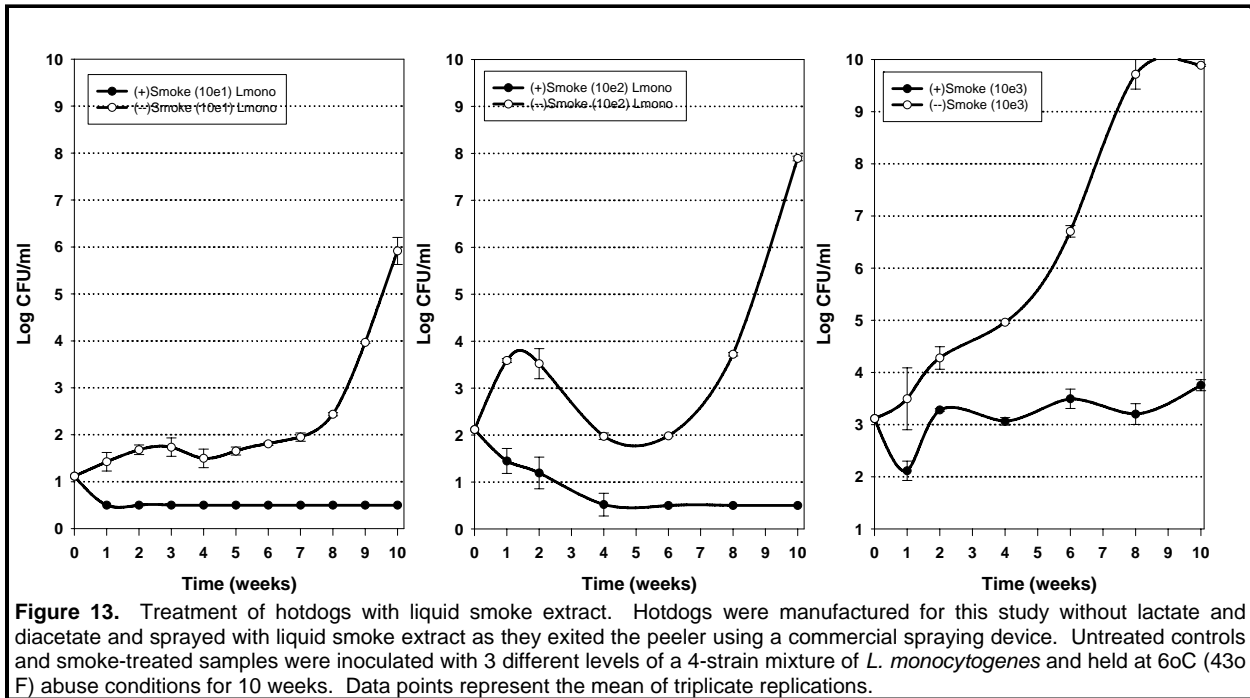
We have used liquid smoke condensates alone (Zesti-B) as effective surface treatments to reduce and prevent the growth of *L. monocytogenes* on hotdogs (Fig. 12). Our data showed that liquid smoke extract was effective in reducing/eliminating *L. monocytogenes* with as short as a 5-sec dip time (Fig. 12A). In subsequent experiments, we have proceeded to examine the shortest possible treatment times, using 1-sec dip and spray regimens (Fig. 12B).



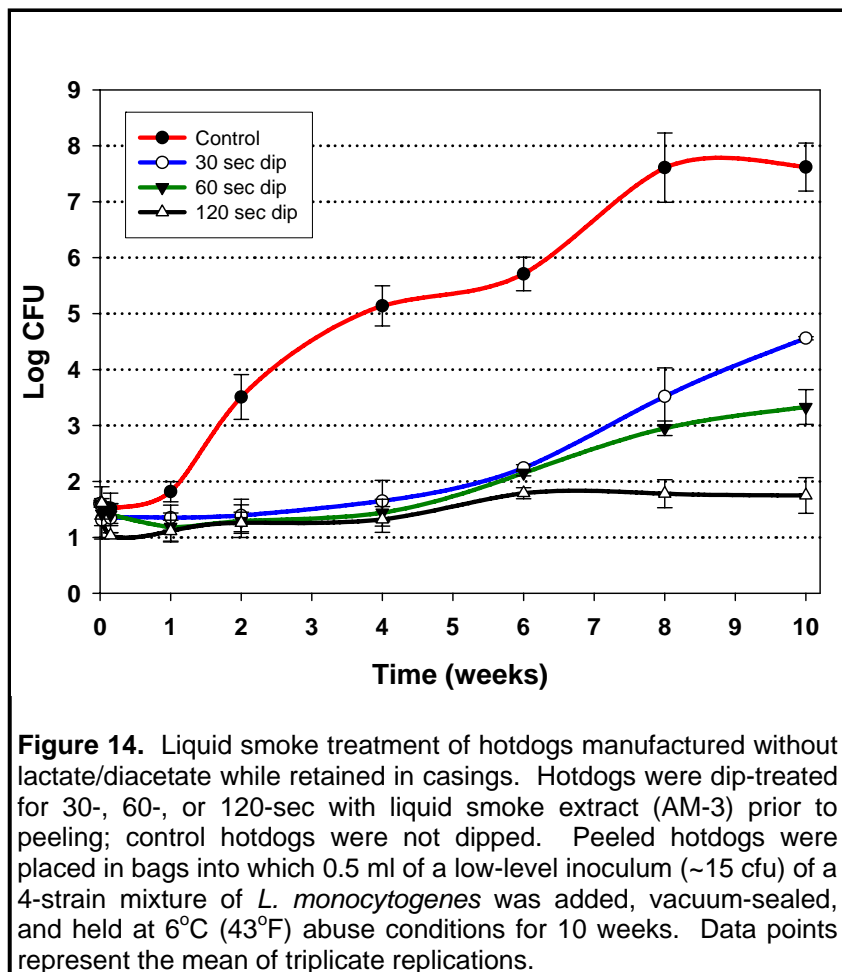
The above data represents some of our earlier work with liquid smoke shelf life experiments whereby we used low shelf life temperatures (i.e., 35°F). However, in subsequent shelf life assays, we used higher temperatures (i.e., 43°F) in order to demonstrate growth, and control, at higher abuse temperatures. The data above (Fig. 12) demonstrates the effective reduction (of low levels of *Listeria*) and prevention of outgrowth on hotdogs during 10-weeks of shelf life.

We have also worked closely with Bar-S Foods to test commercially-prepared and sprayed hotdogs using their formulation when lactate/diacetate were not added. Hotdogs tested in Fig. 13 uses hotdogs (without lactate/diacetate) that were sprayed with a commercial sprayer upon exit at the peeler, packaged, stored, and subsequently repackaged with the challenge inoculum and held at 43°F for shelflife testing. We examined the affect of 3 different inoculation levels ( $10^1$ ,  $10^2$ , and  $10^3$  CFU/ml) on both control and Zesti-B-treated hotdogs, both without lactate/diacetate. The liquid smoke was effective in reducing and preventing outgrowth at all 3 inoculation levels. When using high levels of inoculum (i.e.,  $10^3$  cfu; Fig. 13C), *Listeria* growth was prevented but the organism was not completely eliminated. In hindsight, additional testing may identify the optimal commercial spray treatment regimen as the high velocity of the hotdog exiting the peeler may not have allowed sufficient liquid smoke to be retained on product. Also, the fact that hotdogs were packaged, transported to our lab, and then repackaged upon inoculation could have reduced the amount of residual liquid smoke that normally would have been associated with product that remained in the original package.



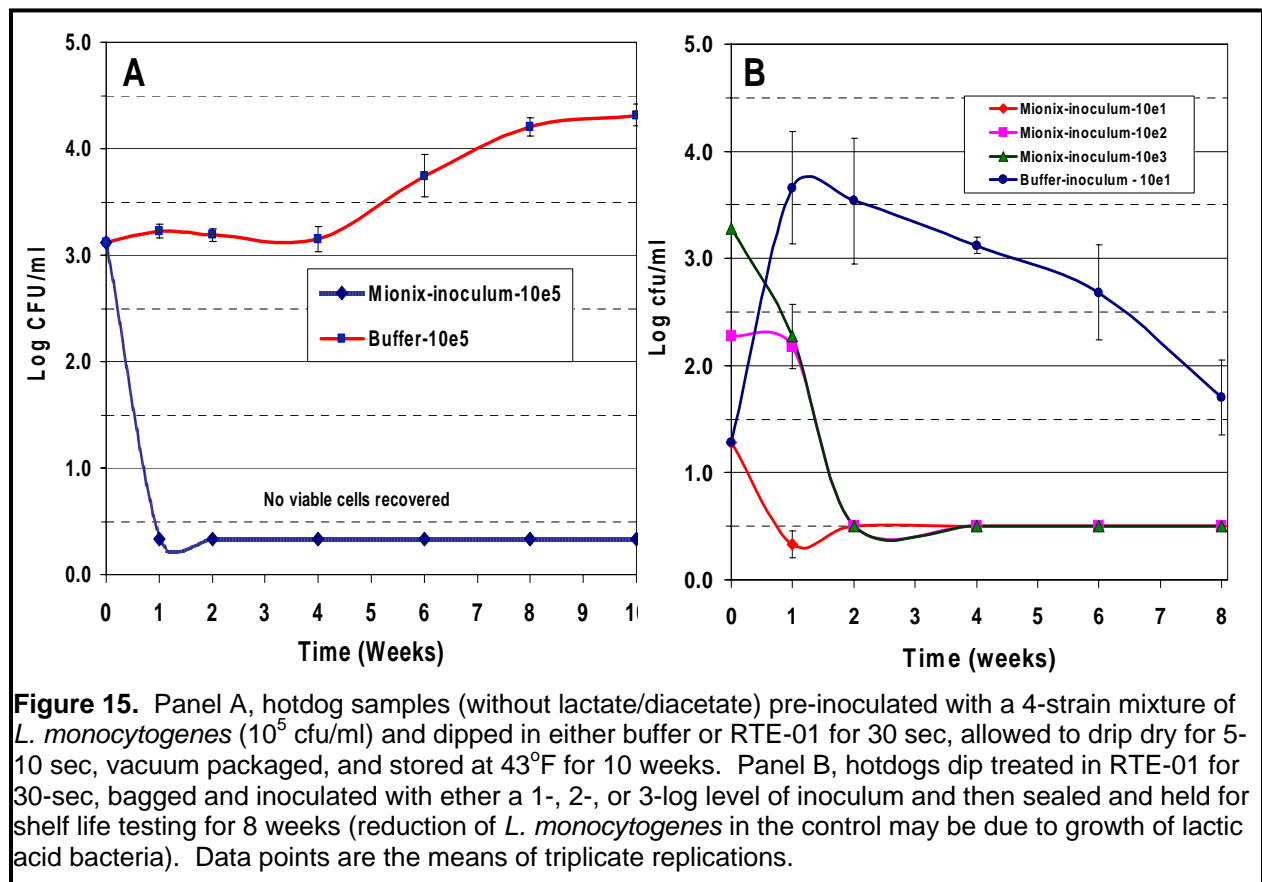


We also examined the effect of pre-treatment of hotdogs in casings in liquid smoke. Hotdogs manufactured without lactate/diacetate were dipped for 30-, 60-, or 120-sec in liquid smoke condensate (AM-3), peeled, bagged and inoculated with approximately 15 cfu *L. monocytogenes*, and subjected to shelf life testing for up to 10 weeks in comparison to untreated control hotdogs (Fig. 14). Untreated control samples demonstrated a 2-log increase in *L. monocytogenes* within 2 weeks resulting in up to a 7.6-log level by the end of the 10-week shelf life period. Dipping of encased hotdogs for any of the dwell times we used prevented growth of *L. monocytogenes* for up to 4 weeks before increasing while dip treatment of 120 sec prevented growth for the entire 10-week period. Such treatments could be done as either part of a post-process chill regimen, or to 'sanitize' the casings with an antimicrobial prior to peeling. Casing waste

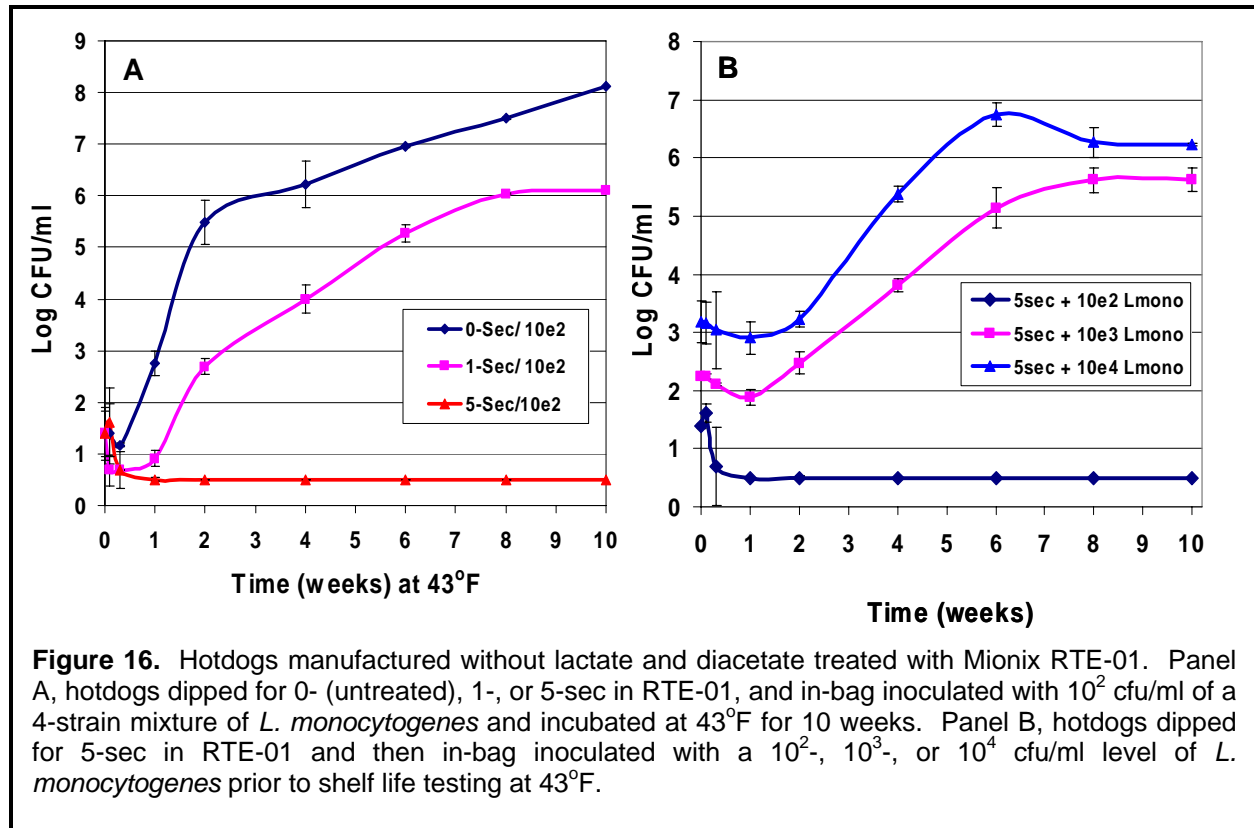


bins have some of the highest incidence for *L. monocytogenes* because they collect casings from an entire batch of hotdogs that were subject to prior handling and which may collect at the peeler equipment surface itself. Treatment of encased hotdogs could result in both reduction of *L. monocytogenes* that could collect at the peeler as well as contribute to prevention of growth of *L. monocytogenes* during the shelf life of product by leaching through the cellulose casings. Although the treatment employed here on encased product may be considered 'long' or impractical for some processors, it could be shortened and combined with additional application on peeled product downstream in the process, prior to packaging. The effective permeation of hotdog casings in such a short time is indicative of the small molecular weight of the active components often thought to be the phenolics associated with liquid smoke.

**Mionix RTE-01.** Mionix manufactures an antimicrobial solution (SAFE<sub>2</sub>0 RTE-01) consisting of lactic acid and acidified calcium sulfate. We examined the product application to hotdogs in several approaches. In one approach, we applied the solution by dipping for 30-sec either after inoculation (Fig. 15A) or before inoculation (Fig. 15B) in comparison to control and for shelf life analysis for 10 weeks. For pre-inoculation of hotdogs prior to dip treatment, we used a high level inoculum ( $\sim 10^5$  cfu/ml) to treat hotdogs before dip treatment. As expected of surface contaminants that may be dipped in an acid solution, there was a quick and effective reduction of the inoculum in which outgrowth was prevented for the duration of the shelf life testing (Fig. 15A). Tests with shorter dip treatments were not as effective (data not shown). We also examined pre-treatment by dipping (30-sec) in RTE-01 followed by inoculated challenge at 3 different inoculation levels. The long dip time still had effective control on reducing the inoculum and preventing outgrowth for 8 weeks (Fig. 15B).



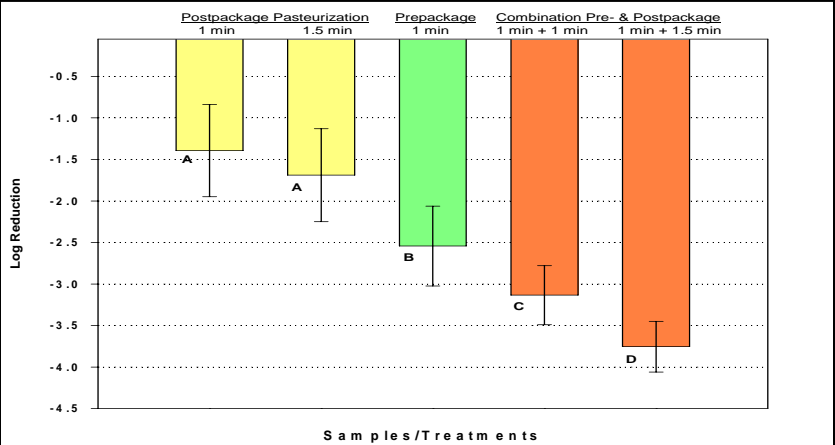
Encouraged by the data obtained above, we examined shorter dip times of 1- and 5-sec with RTE-01 followed by inoculation at the  $10^2$  cfu level with *L. monocytogenes* (Fig. 16A). Although 1-sec was too short to prevent outgrowth of *L. monocytogenes* during the shelf life study, a 5-sec treatment was capable of both, inhibiting and preventing growth of *L. monocytogenes*, when inoculated at the  $10^2$  cfu level, which is likely at the high-end level of what could be obtained by in-plant contamination of product. Based on this data, we examined a 5-sec dip with increasing levels of inoculum at the  $10^2$ ,  $10^3$ , and  $10^4$  cfu/ml level and found the limit for a 5-sec dip in RTE-01 was a post-process (and post-RTE-01 treatment) contamination event limited to  $10^2$  cfu (Fig. 16B). was able to prevent outgrowth for 10 weeks (data not shown). The data presented here indicate that effective regimens can be obtained with RTE-01 with relatively short treatment time at or above 5-sec for what would be considered practical levels of in-plant contamination.



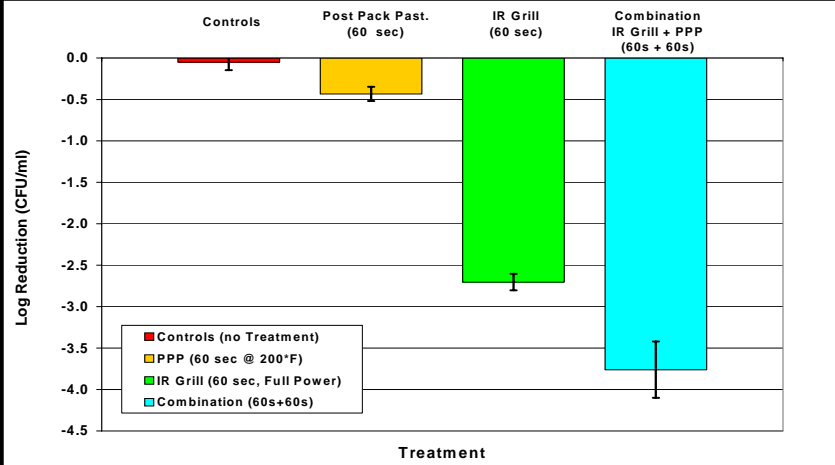
**B. Pre- and Post-package pasteurization of large RTE processed meats:**

**1. To examine the effectiveness of the combination of pre- and post-package pasteurization of RTE deli turkey**

The combination pre- and post-package pasteurization methods capitalize on removing negative aspects of each process. With prepackage pasteurization, there is a concern for getting the product packaged quickly after radiant heat surface pasteurization for fear of recontamination. For post-package pasteurization, extended heating periods to obtain significant reductions generates purge. With the two processes together, there is additional processing after packaging the product eliminating the potential for recontamination and the minimal time spent for postpackage pasteurization (starting with a warm surface rather than chilled) reduces or eliminates the generation of purge. The combination of these processes for the conditions listed is capable of up to nearly a 4-log reduction of *L. monocytogenes*.



**Figure 17.** Reduction of *L. monocytogenes* by postpackage-, prepackage-, and combination pre- and post-package pasteurization of deli turkey. Treatments were 60-sec pasteurization in water (200°F) and/or 60-sec residence time in a radiant heat oven (air temperature ~500°F at product surface).



**Figure 18.** Reduction of *L. monocytogenes* by postpackage-, prepackage-, and combination pre- and post-package pasteurization of deli roast beef. Treatments were 60-sec pasteurization in water (200°F) and/or 60-sec residence time in a radiant heat oven (air temperature ~500°F at product surface).

**2. To examine the combination of pre-, or postpackage pasteurization, of RTE deli meats in combination with antimicrobials and impact on outgrowth of *Listeria monocytogenes***

See section A, #2 above.