USE OF HOT WATER SHRINKAGE OF COOKED HAM TO CONTROL LISTERIA MONOCYTOGENES

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Abstract – This paper aimed to evaluate post-packaging hot water immersion as an effective intervention against *Listeria monocytogenes* in the cooked ham process. Triplicates of ham slices (10x10 cm, 1 cm thickness) were inoculated with 10^5 CFU/cm^2 of Lm, vacuum packaged and placed in a hot water bath at 75, 80, 85, and 90 °C for 0 (control), 20, 25 and 30 s. Post-treatment negative samples were subjected to a 48-h selective enrichment in order to detect sub-lethally injured cells. Holding ham slices for 30 s in hot water at 75, 80, and 85 °C caused limited reductions of 0.26, 0.32, 1.01 log CFU/cm^2 of the pathogen, respectively. After hot water immersion at 90 °C for 30 s *L. monocytogenes* was not detectable. Enrichment data on samples from this treatment showed no survival of sub-lethally injured cells. The results indicate that short-term hot water immersion could be an effective intervention strategy to control Lm in the post lethal environment of the cooked ham process. However, the product should be held at $\geq$ 90 °C for a minimum of 30 s. Information from this study would be useful for ham processors to evaluate current practices and provide consumers with safer foods.

Key Words – Hot water immersion, Listeria monocytogenes, post lethal intervention.

I. INTRODUCTION

Cooked ham is a Ready-To-Eat (RTE) product that supports the growth of *Listeria monocytogenes* [1, 2]. Hot water tanks are widely used in Mexico for the shrinkage of vacuum packaged meat products. Most establishments use water at 60 to 90 °C and hold the product just for a few seconds. Perhaps, the short retention time at this stage has led companies to rely more on the inclusion of anti-listerial agents in product formulation, as well as on more expensive post pasteurization processes. However, since post lethal contamination with the pathogen occurs in the surface of the ham, short-term hot water immersion may also be useful to meet the food safety objectives for *L. monocytogenes*. It is also likely to be cheaper than post process pasteurization, which requires holding the product in the oven for a relatively long time. Therefore, the objective of this study was to evaluate post-packaging hot water immersion as an effective intervention against *L. monocytogenes* in the cooked ham process.

II. MATERIALS AND METHODS

A *L. monocytogenes* strain donated by the National School of Biological Science of the National Institute of Technology was used for this study. The strain was biochemically characterized and kept refrigerated in mineral oil prior to conducting the experiment. From the stock culture a pre-culture in Brain Hearth Infusion (Merck®) of 24 h at 30 °C was performed. From the latter culture (ca. 10^8 CFU/ml) 200 µL were used for sample inoculation. Triplicates of ham slices (10x10 cm, 1 cm thickness) were aseptically prepared in the laboratory from ham pieces bought at a local store. The slices were inoculated with the *L. monocytogenes* strain at a level of 10^5 CFU/cm^2. A negative uninoculated control was also included in order to quantify native *Listeria spp.* that may be present in the product. The inoculum was evenly spread over the surface of ham slices with a sterile glass spreader. The samples were then vacuum packaged and equilibrated for 30 min at 4 °C before hot water immersion at 75, 80, 85, and 90 °C for 0 (control), 20, 25 and 30 s. The temperature of the samples was monitored by means of a thermocouple probe that was sealed in

the plastic bag, using the uninoculated control referred above. The ham slices were not refrigerated immediately after heat treatment in order to simulate industry conditions. After heat treatment, 100 ml of buffered Listeria enrichment broth (Merck ®) were poured in the bag containing the ham slices. The bags were shaken vigorously for 1 min and afterwards, 1 ml of the broth was pipetted and serially diluted (up to 10^{-5}). From each dilution a 1 ml aliquot was pipetted, then poured into plates containing Oxford selective media with supplement (Merck®) and finally spread with a sterile glass spreader, following the Bacteriological Analytical Manual methodology [3]. The plates were incubated at 30 °C and the results were read after 24 and 48 h of incubation. The detection limit of the method was 1 log CFU. Heat-treated negative samples were recorded as 1 log CFU for the purpose of data analysis. These samples were also subjected to a 48-h selective enrichment in order to detect sub-lethally injured cells. The effect of holding time within each temperature on *L. monocytogenes* survival was tested for significance by means of a one-way analysis of variance.

III. RESULTS AND DISCUSSION

Mean maximum surface temperatures of ham slices varied between 60.3 and 75.5°C across treatments (Table 1). As expected, the longer the holding times at each treatment, the higher the surface temperatures of the ham. In general, the surface temperatures reached at 75 and 85 °C did not seem to be high enough as to have a significant lethal effect on *L. monocytogenes.*

Table 1. Effect of holding time during hot water shrinkage on the ham surface temperature (n=3)

Temperature, °C	Holding time, s			
	20	25	30	SE[1] ±
75	60.3[b]	62.5[a]	64.2[a]	0.85**
80	64.8[c]	67.3[b]	70.5[a]	0.88***
85	68.3[c]	70.4[b]	72.3[a]	0.75***
90	70.2[c]	73.6[b]	75.5[a]	0.31***

[1]Standard error of estimation

[a, b, c, d] Means with different superscripts in the same row are significantly different (P<0.05)

**P<0.01; ***P<0.001

The latter was confirmed by the fact that holding ham slices up to 30 s in hot water at 75 and 80 °C (Table 2) had no effect on the survival of *L. monocytogenes* (P>0.05) and log reductions were insignificant (Table 3).

Table 2. Survival of *L. monocytogenes* (log CFU) in cooked ham (n=3) after hot water shrinkage

Temperature, °C	Holding time, s				
	Control	20	25	30	SE[1] ±
75	4.89[a]	4.67[b]	4.73[b]	4.64[b]	0.08**
80	4.93[a]	4.67[b]	4.54[b]	4.59[b]	0.08***
85	4.92[a]	4.51[b]	4.28[c]	3.90[d]	0.10***
90	4.91[a]	3.09[b]	2.23[c]	< 1[d2]	0.13***

[1]Standard error of estimation

[2]Detection limit of the method was 1 log CFU. Samples held for 30 s at 90 °C were negative to *L. monocytogenes* and were recorded as 1 log for data analysis.

[a, b, c, d] Means with different superscripts in the same row are significantly different (P<0.05)

**P<0.01; ***P<0.001

Treatment at 85 °C did affect the survival of *L. monocytogenes* (Table 2). However, the maximum reduction achieved at this temperature was about 1 log CFU after 30 s of holding time (Table 3).

Table 3. Reductions in *L. monocytogenes* concentration (log CFU) in cooked ham (n=3) after hot water shrinkage

Temperature, °C	Holding time, s			
	20	25	30	SE[1] ±
75	0.24	0.18	0.26	0.08
80	0.24	0.37	0.32	0.08
85	0.40[c]	0.63[b]	1.01[a]	0.10***
90	1.82[c]	2.67[b]	3.91[a2]	0.13***

[1]Standard error of estimation

[2]Detection limit of the method was 1 log CFU. Samples held for 30 s at 90 °C were negative to *L. monocytogenes* and were recorded as 1 log for data analysis.

[a, b, c, d] Means with different superscripts in the same row are significantly different (P<0.05)

***P<0.001

Results suggest temperatures $\leq$ 85 °C in the hot water shrink tank are not suitable for meeting the food safety objectives of *L. monocytogenes* (e.g. <

0.04 CFU/g or non-detectable). Since a concentration of 1-1.7 log CFU for this pathogen in the finished product has been reported [4, 5] any effective treatment should provide at least a 3-log reduction in order to meet the food safety objectives. The use of longer holding times at temperatures of 85 °C or lower may not be as attractive to meat processors due to a negative effect on the plant's productivity. Hot water shrinkage at 90 °C also had a significant effect ($P<0.05$) on *L. monocytogenes* survival (Table 2). The pathogen was not detected in samples from this treatment after 30 s of holding time. Selective enrichment of negative samples did not show growth of sub-lethally injured cells after 48 h of incubation. Therefore, treatment at 90 °C for 30 s in the hot water shrink tank may yield about 5 log reductions in the concentration of *L. monocytogenes*. The latter effect may be enough to control post lethal contamination scenarios of 2 to 3 log CFU of the pathogen. Thus, operation of the hot water shrink tank at 90 °C for a minimum of 30 s may work well under industry conditions as an effective post lethal intervention against *L. monocytogenes* in the cooked ham process. The relatively short holding time (30 s) at 90 °C may be feasible – from a productivity standpoint – in many meat processing establishments.

IV. CONCLUSION

Hot water shrinkage of packaged cooked ham seems to be a potential alternative to meet the food safety objectives for *Listeria monocytogenes.* This process step may be included as a post lethal critical control point for the cited pathogen in the HACCP plans of processing establishments.

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