University of Alberta

Validation of a post-packaging pasteurization process to eliminate *Listeria monocytogenes* from ready-to-eat meat products

by

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Abstract

A small steam and hot water pasteurization unit was validated for its effectiveness in the elimination of *Listeria monocytogenes* on ready-to-eat meat products. Bologna, turkey breast and roast beef slices, and smoked sausages were inoculated with a *L. monocytogenes* cocktail and pasteurized to internal temperatures of 60°C, 65°C, 70°C, 75°C, and 80°C. Products were shingled packaged and sampled at three different areas to determine thermal processing for each section. A 5 log CFU/g cell count reduction was achieved during pasteurization of bologna to 75°C, turkey breast slices to 80°C, and roast beef slices to 70°C, regardless of the area sampled. Turkey breast and bologna exposed to heat on both sides had greater cell count reduction, but roast beef among the different areas sampled were not significantly different. Purge produced in the packages of pasteurized sliced bologna was significantly less ($P<0.05$) than turkey breast and roast beef for all pasteurization temperatures.

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List of Symbols and Abbreviations

Atm	atmosphere
CFU	colony-forming units
CFIA	Canadian Food Inspection Agency
D	decimal reduction time
FDA	Food and Drug Administration (USA)
FSIS	Food Safety Inspection Service
LM	*Listeria monocytogenes*
min	minute
N_0	initial population
N_1	final population
PPP	Post-packaging pasteurization
RH	relative humidity
RTE	Ready-to-Eat
s	second
SD	Significantly different
SEM	Standard Error of the Mean
x *g*	times gravity
TNTC	Too numerous to count
USDA	United States Department of Agriculture

Chapter 1: Literature review and Introduction

1.1 Food safety

The Centre for Disease Control and Prevention (CDC) reports that "roughly 1 in 6 Americans get sick and 3000 die of foodborne diseases annually" (CDC, 2011) and it is estimated that foodborne illness costs the United States $152 billion a year factoring in medical expenses and loss of productivity (Produce Safety Project, 2010). There are seven pathogens that cause the majority of foodborne illness: *Salmonella*, norovirus, *Campylobacter*, *Toxoplasma*, *Escherichia coli* O157, *Listeria monocytogenes* and *Clostridium perfringens* (CDC, 2010). In Canada, it is estimated there are 11 million cases of foodborne illnesses each year (CFIA, 2011). In 2008, a major outbreak of listeriosis associated with products from Maple Leaf Foods Inc. resulted in 57 confirmed cases and 23 deaths costing the company $27 million in law suits (Public Health Agency of Canada, 2010; CBC, 2008). Consumer concerns about food safety and quality include aspects such as food additives/preservatives for processed foods, foodborne pathogens or contamination, and product freshness (Agri-Food Canada, 2005). A recent poll reported that "76% of consumers are more concerned about the safety of their foods now than five years ago" (Deloitte, 2008) and "57% of consumers have stopped eating a food produced by a company due to a recall" (Deloitte, 2008). Many consumers do not trust food companies to produce and sell healthy and safe food products (IBM Research, 2009). However, a recent survey

(Léger Marketing, 2011) found that Canadians are becoming more confident in food safety as confidence ratings increased in 2011 compared to 2010 and 2008.

1.2 Ready-to-eat meat products

Two major factors that influence the purchase of ready-to-eat (RTE) meats by female senior citizens and those of childbearing age and individuals who are susceptible to infection with *L. monocytogenes* include the fat content and price of the product (Lenhart et al., 2008). For young women, factors such as product appearance, sodium content, and nitrite content play a role in the purchase intent for RTE products. For seniors, fat content was a major quality concern. Two-thirds of households store open packages of deli meats in their refrigerators longer than the USDA-recommended guidelines (Cates et al., 2006). A web based survey of consumer behavior found that 32% of consumers felt date of packaging was the most important factor in their decision to purchase a refrigerated food product, whereas 29, 24 and 8% of consumers thought smell, appearance and taste, respectively, are the most important factors (Kosa et al., 2007a). In total, 61% of consumers indicated that they rely on their senses to determine food safety, which is an unsafe practice as foods contaminated with *Listeria* do not smell, taste, or appear any different from uncontaminated products. RTE deli meats such as bologna, roast beef, turkey breast, and sausages typically have a high content of sodium or fat (Heart and Stroke Foundation, 2009), but as consumers become more health conscious, products with lower sodium and fat are in more demand (Agriculture and Agri-Food Canada, 2005).

1.3 *Listeria monocytogenes*

L. monocytogenes is a Gram-positive, facultative anaerobe that is ubiquitous in the environment and can be found in soil, water, sewage (Farber & Peterkin, 1991).The organism is capable of survival between -1 and 50°C, in the pH range of 4.6-9.0, and at high salt concentrations and low oxygen environments (ICMSF, 1996). The symptoms of listeriosis, the infection caused by *L. monocytogenes*, is mild in healthy individuals, with symptoms including nausea, vomiting, diarrhea, headaches, and fever. The infective dose for healthy individuals is not known, but is thought to be around 10^9 CFU/g product (Farber & Peterkin, 1991). The most susceptible demographic are immunocomprised, elderly, or pregnant individuals. If individuals in this demographic are not properly treated, symptoms can be severe resulting in septicemia, meningitis, encephalitis, and spontaneous abortions (Vazquez-Boland et al., 2001). The infective does for this group can be as low as 100 cells (Hui, 2005). The growth of *L. monocytogenes* in a food does not result in changes in product odour, flavour or appearance.

There are many interventions to minimize pathogen contamination and improve food safety, but for *L. monocytogenes* in RTE meat products it is important to either prevent post-processing contamination or subject the product to a lethal step that will eliminate contamination on products and inhibit the growth of surviving cells (Sofos, 2008; 2009a). Post-processing interventions are important if the product becomes contaminated and the product allows growth of the pathogen during the storage. In 2003, the Food Safety and Inspection Service

(FSIS) in the USA instituted new guidelines with the aim to control *L. monocytogenes* on RTE meats. The guidelines include the following alternatives for processors. Alternative (i) includes application of a post-lethality treatment (may be an antimicrobial agent or process) that reduces or eliminates *L. monocytogenes* on the product *plus* an antimicrobial agent or process that suppresses or limits growth of the pathogen. Alternative (ii) includes the application of a post-lethality treatment that reduces or eliminates *L. monocytogenes* on the product *or* an antimicrobial agent or process that suppresses or limits growth of the pathogen. Alternative (iii) is a combination of verified sanitation procedures and microbiological testing program (FSIS, 2003). One of these three alternatives must be selected by processors to control the presence or growth of *L. monocytogenes* on RTE meat products. Any Canadian processors that are shipping product to USA destinations must comply with these guidelines.

Health Canada's policy for *L. monocytogenes* on RTE meats indicates that *L. monocytogenes* should be absent in 125 g (5 sample units of 25 g product) after enrichment (Health Canada, 2011). The Canadian Food Inspection Agency's guidelines on *L. monocytogenes* in RTE foods requires that for an antimicrobial agent to be acceptable, the agent "allows no more than 2 log CFU/g increase in *L. monocytogenes* throughout the stated shelf-life of the product" (CFIA, 2011) and that a "post-lethality treatment is considered satisfactory when it achieves at least 3 -log reduction in number of *L. monocytogenes*" (CFIA, 2011).

1.3.1 Sources of *Listeria* spp.

In the processing facility, *Listeria* can be spread by contaminated workers as a result of improper worker hygiene. Personal protective equipment can also be a source of contamination such as aprons, gloves, and lab coats. The organism can be found in different locations in the plant such as coolers, drains, still water tanks, conveyor belts as well as the equipment used to prepare RTE meat products such as slicers, cutters, and packagers (FDA, 2009). Cutters and slicers can be sources of contamination because the equipment can often be difficult to clean and the *Listeria* can adhere to various surfaces and form biofilms. Biofilms are an extracellular polysaccharide matrix that protects the organism from cleaners and sanitizers and provides greater resistance to antimicrobial agents (Møretrø and Langsrud, 2004). Biofilms may be composed of single strains or mixed cultures (Sofos and Geornaras, 2010). Biofilms can adhere to floors, walls, pipes, drains, in conveyor belts, pasteurizers and different surfaces such as stainless steel, aluminum, nylon, Teflon, rubber, and plastic (Sofos, 2009b). When bacteria first adhere to a surface the process is reversible, but cells eventually form microcolonies and multilayers biofilms (Møretrø and Langsrud, 2004). Disinfectants are less effective on thick layer biofilms compared to ones that are a thin layer and are less effective on older biofilms than newly formed ones. *L. monocytogenes* in multi-species biofilms are more resistant than when they are present in a biofilm of a single strain of *L. monocytogenes* (Frank and Koffi, 1990). *L. monocytogenes* can persist from months to years in a food processing facility (Tompkin, 2002). Biofilm formation is important as a persistent source of

contamination of RTE meat products if proper sanitation procedures are not implemented.

1.4 Thermal resistance

Typically, *L. monocytogenes* does not survive thermal treatment and adequate cooking will eliminate the organism. When *L. monocytogenes* is exposed to sublethal temperatures, the organism responds to the environmental stress and synthesizes heat shock proteins (Farber and Brown, 1990), which increase its overall thermal resistance. Therefore, if *Listeria* is exposed to sublethal temperatures between 44°C and 48°C (Farber and Peterkin, 1991) before a higher final temperature, the organism will have a higher heat resistance. Graphically, this effect can be seen by the tailing or shouldering effects on a survivor curve (McCormick et al., 2003). After a heat shock caused by growth at a high temperature, *Listeria* can maintain an increased thermotolerance during growth in meat stored at refrigeration temperatures for 24 h (Farber and Brown, 1990). An increase in holding time at high temperatures also results in greater heat resistance in *Listeria* (Farber and Brown, 1990). A fast rate of heating (heat to 60°C at > 5°C/min) does not induce thermotolerance, but at slow rates (0.7°C/min), thermotolerance increases proportionally as heating rates are decreased (Stephens et al., 1994). The direct mechanism for an adaptive response to heat and the relationship between heat shock proteins and higher thermotolerance is not fully known (Sergelidis and Abrahim, 2009). This could be a problem in the RTE meat industry if products are contaminated with *L.*

monocytogenes, temperature abused (exposed to sublethal temperatures above the optimal growth temperature), which allows the organism to become even more heat resistant. Strain variation, previous exposure to heat shock, growth conditions (i.e. temperature, growth medium) also factor into differences in thermal resistance (Doyle et al., 2001). *L. monocytogenes* in the stationary phase are sometimes more heat resistant than those in the exponential log growth phase (Doyle et al., 2001) and the composition of the heating menstruum (carbohydrates, proteins, lipids, and solutes), water activity (a_w), pH, added preservatives, and method of heating are important (Juneja et al., 2011; O'Bryan et al., 2006). For example, the D-value for *L. monocytogenes* in lean ground beef (2% fat) was lower than that determined in a high fat (30.5%) ground beef (Fain et al., 1991). The addition of salt and spices can have a protective effect as the addition of NaCl increases the overall resistance of *L. monocytogenes* (Bae and Lee, 2010). The addition of salts and spices to sausage meat increased the D-values 4- to 5-fold compared to the D-values determined in product without the additives (Faber et al., 1988). When validating and optimizing a pasteurization process for RTE products, the fat and sodium content of individual products must be taken into consideration.

1.5 Post Packaging Pasteurization

Post-packaging pasteurization (PPP) may be a low cost alternative to high pressure processing for eliminating populations of *L. monocytogenes* from packaged RTE deli meats. To determine the effectiveness of a submersion water

heating system to eliminate *L. monocytogenes* inoculated onto different RTE deli meats, packaged RTE meat samples were submerged into a water bath system set at 90.6°C, 93.3°C, and 96.1°C for times ranging from 2 to10 min (Muriana et al., 2002). These processes produced a 2- to 4-log reduction in numbers depending on the product (Muriana et al., 2002). The thermal processing resulted in excess purge in roast beef and formed turkey products resulting in lower microbial reductions compared to reductions achieved in drier products (hams and smoked turkey). The appearance of purge is unappealing to consumers which is a major concern when using a PPP process. Continued work by Muriana et al. (2004) observed that a submerged water PPP system achieved 1.95 to 3 log CFU reductions of *L. monocytogenes* inoculated onto deli turkey when processed at 93.3°C for 2, 3, 4, or 5 min. Processing time depended on the manufacturer of the product (different processing) resulting in different reductions in numbers of *L. monocytogenes*. However, the PPP process only heated the outer 1 cm of the surface that led to a minimal effect on product appearance and quality (Muriana et al., 2002). Though appearance is important, it is possible that if processing equipment used to prepare RTE meat products were contaminated with *L. monocytogenes* then the entire product within the package could be contaminated. If a process only heated the outer surfaces, it could potentially allow survivors of *L. monocytogenes* on inner slices. When fully cooked chicken breast strips (454 g sample) were inoculated with *L. innocua* and placed in a water cooker to be processed at 88°C, it took 25 min to achieve a two log reduction and 35 min to achieve a seven log reduction (Murphy and Berrang, 2002). Thickness of RTE

bologna has an effect on heating rates for PPP processes. Bologna slices of different thicknesses (4, 12, and 20 mm) were tested in a hot water bath set at temperatures ranging from 60 to 90°C and the thicker pieces as well as product with a higher fat content had slower heating rates (Mangalassary et al., 2004). Due to the heat sink effect, the package's surface is first heated by conduction and the heat then diffuses towards the interior by convection. During the diffusion of heat towards the interior, there is a loss in surface heat. The thicker the product, the more pronounced the effect. Eventually, given enough time, the entire product accumulates enough heat for the surface and interior parts to reach the same final temperature (Mangalassary et al., 2004). To obtain a 5-log reduction at various temperatures, it took 2.5 to 6 times longer for 20 mm thick samples compared with 4 mm samples with the same percentage of fat (Mangalassary et al., 2004). This is a very important factor to consider when validating a pasteurizer for reduction of *L. monocytogenes* to make certain the interior regions obtain the same thermal exposure as the surface. This is especially true with stacked bologna, smoked sausages, or even shingled product in which the middle slices would be least exposed to the thermal process. Selby et al. (2006) studied the ability of a hot water pasteurization unit to obtain a 3-log reduction of *L. monocytogenes* on inoculated bologna while maintaining the sensory qualities of the product. When a water bath was set to lower temperatures (55°C, 60°C, and 65°C) the times required were impractical to achieve a 3-D reduction. For example, at 55°C, 366 min were required and at 65°C the process took 5 min. The only temperature setting that would be feasible would be at 65°C, as heating at

55, 60, and 62.5°C would take too long for processing of RTE products. Submerging packaged low-fat ready-to-eat turkey bologna inoculated with *L. monocytogenes* into a water bath at 61°C for up to 10 min resulted in detection of cells ($>10^2$), but when the water bath was set to 85°C, no *L. monocytogenes* were detected ($<10^2$) after 10 s of exposure (McCormick et al., 2003). These researchers showed that inactivation of *L. monocytogenes* was possible using an in-package thermal pasteurization process (McCormick et al., 2003). A combination of pre-package pasteurization using radiant surface heating and post packaging pasteurization by water submersion lead to minimal production of purge (Muriana et al., 2004).

1.6 Survival of *L. monocytogenes* after storage

Another important factor to consider when validating PPP processes is the survival of *Listeria* during storage. The industry needs to consider how the consumer will store RTE meat products, the temperature of storage and the length of time a consumer will store a package before consuming the product. Typically refrigerators should be set at 4°C or below (CFIA, 2010); however, not all refrigerators are set to this guideline and some might be even warmer than the temperatures suggested in the CFIA guideline (Gill et al., 2002). Temperatures above 4°C would allow any surviving *L. monocytogenes* to recover and grow. Methods to inhibit the growth of *Listeria* during storage include the addition of antimicrobial compounds. The use of sodium lactate, acetate, or diacetate in frankfurters at the highest allowable concentration, inhibits the growth of *L.*

monocytogenes for 20 to 70 days at 4°C (Bedie et al., 2001). A combination of these antimicrobials controlled the growth of *L. monocytogenes* in refrigerated beef and frankfurters (Mbandi and Shelef, 2001; Samelis et al., 2002). The addition of 3.3% sodium lactate to frankfurters delayed the lag time of *L. monocytogenes* by two weeks (Choi and Chin, 2003). The length of refrigerated storage after purchasing a RTE meat product is dependent on the consumer, thus it is crucial that any PPP process eliminate *L. monocytogenes* or sufficiently reduce the numbers so that during storage *L. monocytogenes* does not recover to above 100 CFU/g. On sliced turkey breast, even with an initial load of 0.04 CFU/g (detection limit of 1 cell/25g), with storage under temperature abuse conditions (8°C) a 100 fold increase would occur in less than 60 days (Pal et al., 2008). It is important to ensure that no survivors exist, as they will have the opportunity to grow throughout the recommended shelf life of the product and any time that the consumer might store the product before consumption.

1.6.1 Refrigeration Conditions

Temperatures in refrigeration units can vary and the top shelf, bottom shelf, and areas closest to the door of the refrigeration unit may not have the same temperature as the rest of the unit. This is especially true for refrigerators in consumers' homes. A nationwide U.S. consumer survey determined that only 11% of respondents owned a refrigerator thermometer to check the temperature within the refrigerator and over one fourth of respondents had refrigerator temperatures above 4.4°C (Kosa et al., 2007b). A third of respondents claimed to

use the internal refrigerator gauges to judge if food is stored safely. Even if a product was free of *L. monocytogenes*, once a package is opened, there is a risk that the product could become contaminated. The USDA and the FDA recommend cleaning refrigerators regularly to reduce bacterial growth (FSIS, 2010).

1.7 Research Objectives

Post-package pasteurization is a potential alternative process to reduce the presence of *L. monocytogenes* on RTE meat products. If the process is effective, the amount of preservatives added to RTE meat products to inhibit growth of pathogens during storage could be reduced making products more attractive for health conscious consumers. As long as the final package remains intact and sealed with the product inside, there is no chance for recontamination.

However, there are factors that must be considered for a PPP process. The first is to determine the type of products suitable for this process. The geometry (size, thickness) or ingredients in a RTE meat product play an important role in validating the effectiveness of the process. Since profit is linked to total production, the ideal pasteurization process requires both reduction of *Listeria*, and fast processing speeds while maintaining product quality. If the process is capable of the first step, but not the second, it may or may not be feasible for a small processing company, depending on the time-to-cost ratio.

The second important factor is evaluation of purge production. During the pasteurization process, moisture is lost from the product resulting in purge

accumulation in the package. Higher pasteurization temperatures result in greater purge formation and products are visibly unappealing to consumers. For an ideal PPP process, the amount of purge formed must be minimized.

The objectives of this research were to:

1. Determine the ability of a small, prototype PPP process to reduce numbers of *L. monocytogenes* by > 3 logs inoculated onto four ready-to-eat meat products: bologna, roast beef and turkey breast slices, and smoked sausages;
2. Determine the amount of purge formed by each product after pasteurization at various temperatures on the day of processing and after four weeks storage at 7°C.

Chapter 2: Materials and Methods

2.1 Product composition and ingredient formulation

The ingredient list for each of the vacuum packaged products used in the study is shown in Table 1. The bologna was obtained from the Food Processing Development Centre (FPDC) of Alberta Agriculture and Rural Development with the calculated values for protein, fat, and sodium content. All other RTE meat products were obtained from meat processors in Edmonton, Alberta. All products were used in experiments within 30 days of receipt. The amount of protein, fat, and sodium for each product was stated on the nutrition label and is shown in Table 2.

Table 1 Product ingredient list for RTE meat products used in this study.

Bologna	Turkey Breast	Roast Beef	Smoked Sausages
Beef, pork, water, modified cornstarch, maltodextrin, sodium phosphate, onion powder, salt, sodium erythorbate, garlic powder, sodium nitrite, spice, propylene glycol (mfg aid)	Turkey, water, salt, dextrose, sugar, sodium phosphate, sodium erythorbate, icing sugar, sodium nitrite, vegetable oil	Beef, water, dextrose, salt, flavouring (soy protein isolate and corn starch), sodium phosphate, hydrolyzed plant protein (corn, soy), tricalcium phosphate, spices, caramel powder (contains sulphites)	Pork, water, mechanically separated chicken, modified corn starch, salt, sugar, soy protein, garlic powder, sodium phosphate, hydrolyzed soy and corn protein, yeast extract, sodium erythorbate, spices, sodium nitrite, smoke

Table 2 Composition of RTE meat products used in this study (obtained from product labels).

Component	Per 100 g of product			
	Bologna	Turkey Breast	Roast Beef	Smoked Sausage
Protein	15 g	21.8 g	16 g	12.5 g
Fat	20 g	1.8 g	4.5 g	17.5 g
Sodium	0.7 g	0.8 g	1.2 g	0.9 g

2.2 Preparation of strains of *Listeria monocytogenes*

Six strains of *L. monocytogenes* were evaluated for use in this study: FS13, FS14, FS15, FS 19, FS 30 (University of Alberta, Edmonton, AB), and ATCC 7644 (American Type Culture Collection; Manassas, VA). Strains with an FS designation were isolated from RTE meat and poultry products (Bohaychuk et al., 2006) and had previously been shown to survive heating at 60°C for 30 min (P.Ward, personal communication). Prior to use in experiments, all cultures were maintained at -80°C in tryptic soy broth (TSB; Difco, Becton Dickinson; Sparks, MD) containing 22% glycerol. A working culture for each strain was prepared by inoculating a loopful of a frozen culture into TSB which was incubated at 37°C for 24 h. From the working culture, a 1% (v/v) subculture was prepared and incubated at 37°C for 24 h before use in experiments. The cell concentration of cultures of the six strains was determined by inoculating working cultures of each strain into 10 mL TBS that was then incubated at 37°C for 24 h. At regular intervals samples were removed and cell counts determined by plating appropriate dilutions onto tryptic soy agar [TSA; 15g/L granulated agar (Difco) added to TSB broth] which were then incubated at 37°C for 24 h. Strains were grown in

triplicate and the final cell concentrations for each strain was averaged. Each strain reached a maximum density between 8.6 and 9.2 log CFU/mL after 24 h of incubation.

2.3 Determination of strain compatibility

A modified deferred inhibition assay was completed to determine competitive inhibition among the strains of *L. monocytogenes* (Fleming et al., 1975). Each culture of *Listeria* was spotted (3 µL) onto All Purpose Tween (APT) agar (Difco), allowed to dry and overlayered with 10 mL soft APT agar (7.5 g/L granulated agar added to APT broth) seeded with a second strain of *Listeria* [1% (v/v)]. Plates were incubated for 24 h at 37°C, and observed for zones of inhibition. All combinations using the six strains were tested for inhibition among strains. Zones of inhibition were visible when FS15 was overlayered on plates spotted with FS13 and FS14; thus, FS15 was removed from the cocktail. The final cocktail used in experiments included *L. monocytogenes* FS13, FS14, FS19, FS30, and ATCC 7644.

2.4 Cocktail preparation and inoculation of meat samples

To produce a cocktail of the five strains of *L. monocytogenes*, 10 mL of each strain was combined into a sterile 250 mL centrifuge bottle and centrifuged at 5000 x *g* for 10 min. The supernatant was decanted and the pellet was washed twice with 50 mL 0.85% (w/v) sterile saline solution. The cell pellet was suspended in 10 mL 0.85% (w/v) sterile saline. Each time a cocktail was

prepared, the cell density was enumerated by plating serial dilutions onto TSA, which was incubated at 37°C for 24 h. The initial concentration of each cocktail was determined to be approximately 6.5 x 10^9 CFU/mL.

To prepare the inoculum, 3 mL of the cocktail was added to 3 L of 0.85% (w/v) sterile saline in a sterile large stainless steel bowl. A sterile stirring rod was used to mix the inoculum, and appropriate dilutions were was spread onto TSA to confirm that the cell concentration in the inoculum was approximately 6.5 x 10^6 CFU/mL.

For inoculation, meat samples were placed into a stainless steel frying basket, and completely immersed into either the cocktail of *L. monocytogenes* or saline solution (uninoculated control) for 30 s with slight agitation to allow uniform inoculation of the product. After shaking off the residual saline, products were placed into sterile stainless steel bowls and allowed to dry inside a level two Biological Safety Cabinet (BK-2-6, Microzone; Ottawa, ON) for approximately 30 min prior to vacuum skin packaging. After drying, the top of the metal bowl was covered with sterile aluminum foil, and the product was transferred to the packaging area.

2.5 Packaging of meat samples

Uninoculated samples were packaged first to avoid cross contamination. For each product, twelve uninoculated and twelve inoculated packages were prepared. Two of the uninoculated and two inoculated packages were not subjected to pasteurization and 10 of each were pasteurized (two for each target

pasteurization temperature). Half of the packages were analyzed on the day of pasteurization and half were stored for four weeks at 7°C. For analysis on the day of pasteurization, samples were immediately placed on ice in a walk in cooler held 4°C. Three replicates were completed for each product.

Samples were packaged using a vacuum skin packager (Intact™ RM571, Koch Equipment LLC; Kansas City, MO) using 8 mil barrier film (203 μm gauge, oxygen permeability of 30 cc/m^2/24 h/atm @ 23°C, 75% RH, and a water vapour transmission rate of 4 g/m^2/24 h/atm @ 38°C, 90% RH, processing temperatures 105°C to 135°C; Ultravac Solutions LLC; Kansas City, MO). The hot plate on the packaging machine was set at 120°C, main vacuum time at 25 s, and first vent time at 0.5 s as per the manufacturer's recommendations for barrier film. For the bologna, turkey breast, and roast beef products, five slices were packaged in a shingled overlapping orientation so that there was one region in which only one side of the product was exposed to the thermal treatment, a second region where both sides of the product were exposed to the thermal treatment, and a third inner region where internal product was not in direct contact with the outer package (Figure 1). These three areas were sampled for determination of microbial counts. Each package was inspected for wrinkles to ensure the vacuum seal was intact. The sealed packages were dipped into a 10% (v/v) bleach solution to remove any potential external contamination, and then dipped in water to remove excess bleach. Packages were labeled and stored at 2°C a minimum of 2 h prior to pasteurization on the same day.

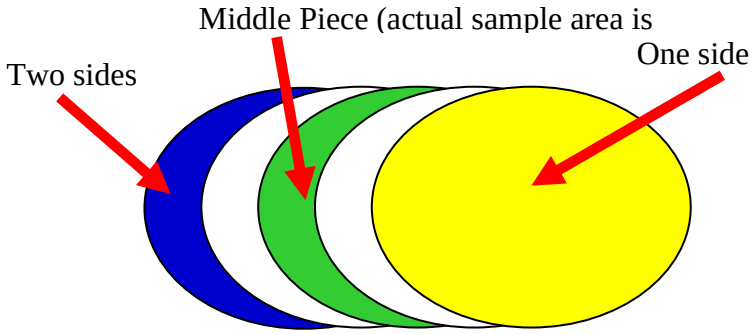

Figure 1 Orientation of meat slices in the package and location of areas used for analysis. The blue slice represents the edge piece, green slice (hidden area) the middle piece, and the yellow slice the one sided piece.

2.6 Effect of conveyor belt speed on pasteurization time

Prior to experiments with inoculated products, experiments were done to determine the speed of the conveyor belt and the corresponding internal temperature range of the sliced bologna, turkey breast, and roast beef products. Pasteurization was done using a Trim Pasteurizer (Stanfos Inc., Edmonton, AB). A conveyor belt moved the product through the Trim Pasteurizer, while steam and re-circulated hot water was sprayed from valves inside the machine. Water levels within the Trim Pasteurizer were adjusted to ensure that samples did not float. The total pasteurization time for each product was recorded from the time the product entered the water spray section of the Trim Pasteurizer (Figure 2), until the product exited the Trim Pasteurizer. For each setting a minimum of 10 packages were tested to ensure accuracy of measurements. Uninoculated samples with a Type K thermocouple (10 foot, 072097B-K, Cole-Parmer; Montreal, QC) attached to a thermometer (600-1010, Barnant, Barrington, IL) inserted into the

centre of the package were used to monitor temperature during pasteurization. The steam was kept at a constant temperature of 85°C, while the speed of the conveyor belt was adjusted to give final internal product temperatures of 60, 65, 70, 75, and 80°C.

To monitor the temperature profile of the sausages during pasteurization, a different method was used. A Type K thermocouple wire attached to a data logger (WD-35427-50, Oakton Eutech Instruments; Vernon Hills, IL) was

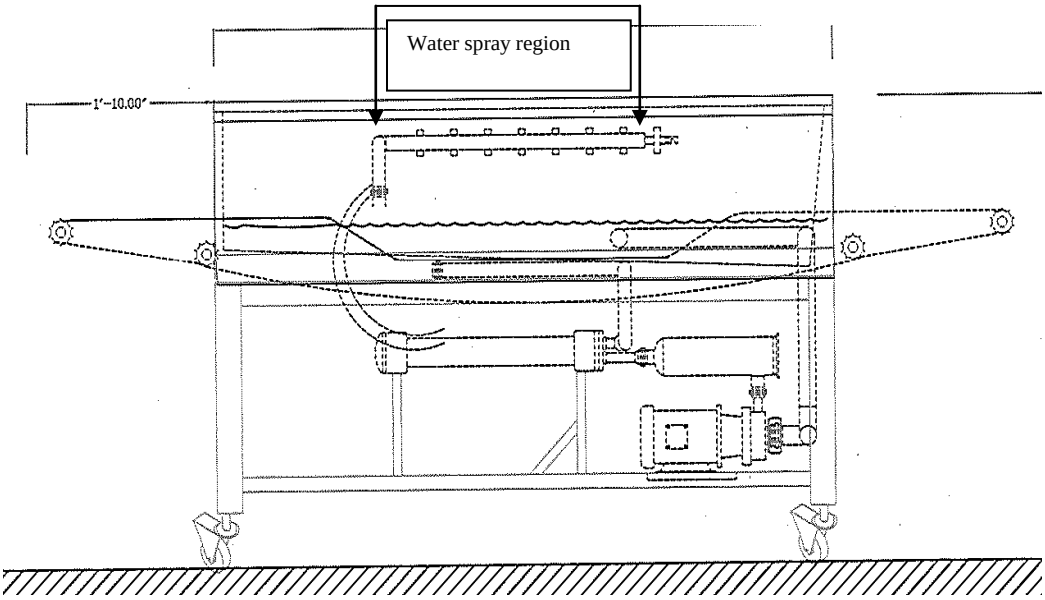

Figure 2 Schematic diagram of the hot water Trim Pasteurizer from Stanfos Inc. The arrows indicate the hot water spray region. Used with permission.

inserted into the package. Four sausages were packaged side-by-side in a single layer, and the thermocouple wire was attached to the product with tape. Probe locations tested included the top surface, the bottom surface, and between the two inner sausages in the package. As the package moved through the Trim Pasteurizer, the data logger recorded temperatures in the package, and allowed for

determination of the time required to reach the desired temperature. Data were manipulated in Microsoft Excel (Microsoft; Redmond, WA)

2.7 Product analysis

Packages of pasteurized and unpasteurized meat products were subjected to analysis on the day of pasteurization and four weeks after pasteurization. Three replicates were done for each product, and duplicate packages were sampled within each replicate for each of the different pasteurization temperatures.

Packages were placed into a Biological Safety Cabinet (BK-2-6, Microzone; Ottawa, ON), and opened using a retractable utility knife treated with 70% (v/v) ethanol. An ethanol-treated knife was used to aseptically cut approximately 10 g of meat from each region of the package (one sided, edge pieces, and middle piece), each sample was placed into a sterile stomacher bag (Fisher Scientific, Edmonton, AB), and 90 mL of 0.1% (w/v) sterile peptone water was added to each bag. The sample was homogenized by stomaching for 1 min, and ten fold serial dilutions were made with sterile 0.1% peptone water.

Appropriate dilutions of homogenized samples were enumerated by spreading aliquots onto the surface of Plate Count Agar (PCA; Difco) for aerobic bacteria, All Purpose Tween (APT; Difco) for presumptive lactic acid bacteria, and Polymyxin Acriflavin Lithium-chloride Ceftazidime Esculin Mannitol agar (PALCAM, Oxoid Limited; Basingstoke, UK) with selective supplement (Oxoid) for *L. monocytogenes. Enterobacteriaceae* were enumerated using a pour-plate technique with Violet Red Bile Agar with glucose [1% (w/w)] agar (VRBG,

Difco). PCA plates were incubated at 30°C for 24 and 48 h, APT and PALCAM were incubated at 37°C for 24 and 48 h, and VRBG plates were incubated at 37°C for 24 h. All agar plates were enumerated after 24 and 48 h and the counts were converted to log CFU/g. Log reduction of *L. monocytogenes*. was calculated using the equation log (N_0/N_1) where Log N_0=log of counts on the unpasteurized sample and Log N_1= log of counts of the pasteurized samples.

Product purge was measured by soaking up the liquid remaining in the package using preweighed paper towels, and weighing the wet towels. The intact package (film, purge, and product) was weighed, the package was opened to remove the product, the inside of the package was wiped using preweighed paper towels, and the empty package was weighed. The amount of purge was calculated by weighing the intact package, and subtracting the product and packaging film. The amount of purge, expressed as the percentage of product, was calculated.

2.8 Enrichment of *L. monocytogenes*.

To determine if *L. monocytogenes* survived pasteurization and recovered during storage, five grams of meat from the middle of each package was added to 45 mL of University of Vermont broth (UVM, Difco), and homogenized by stomaching for 2 min. After incubation of the UVM broth for 24 h at 30°C, 0.1 mL was added to 9.9 mL modified Fraser broth (Oxoid) which was incubated for 24 and 48 h at 37°C. Tubes of modified Fraser broth were inspected after 24 and

48 h for presence/absence of *Listeria*, with black colouration indicating potential positive confirmation. After 24 h incubation, 0.1 mL of each sample was spread onto PALCAM and incubated at 37°C for 24 h. Plates were inspected after 24 h to determine the presence of *L. monocytogenes*. For anomalies between replicates such as the presence of black colonies on PALCAM plates when samples were not expected to contain *L. monocytogenes*, or presence of black colonies from uninoculated pasteurized samples, cells were harvested from the plates and a Gram strain was performed. Cell morphology was used to differentiate between *L. monocytogenes* and *Enterococcus* spp. as both can produce black colonies on PALCAM agar.

2.9 Statistical Analysis

A one-way ANOVA was performed using a mixed model in Statistical Analysis Software (SAS v. 9.3, SAS Institute Inc; Cary, NC) to determine any significant difference in conveyor belt pasteurization times, microbial reductions for the three RTE products, and percentage purge comparing all RTE products on day of pasteurization or after storage. An independent T-test was performed using Statistical Analysis Software to determine any significant difference between percentage purge for each individual RTE meat product on day of pasteurization compared to after storage.

Chapter 3: Results and Discussion

3.1 Verification of experimental parameters

To determine the effect of conveyor belt speed on the time for product to move through the Trim Pasteurizer, the duration of time for product to move through the water spray region to the end of the unit was measured. Statistical analysis determined that there was a significant ($P<0.0001$) difference among pasteurization times at different conveyor belt settings (Figure 3). The time for

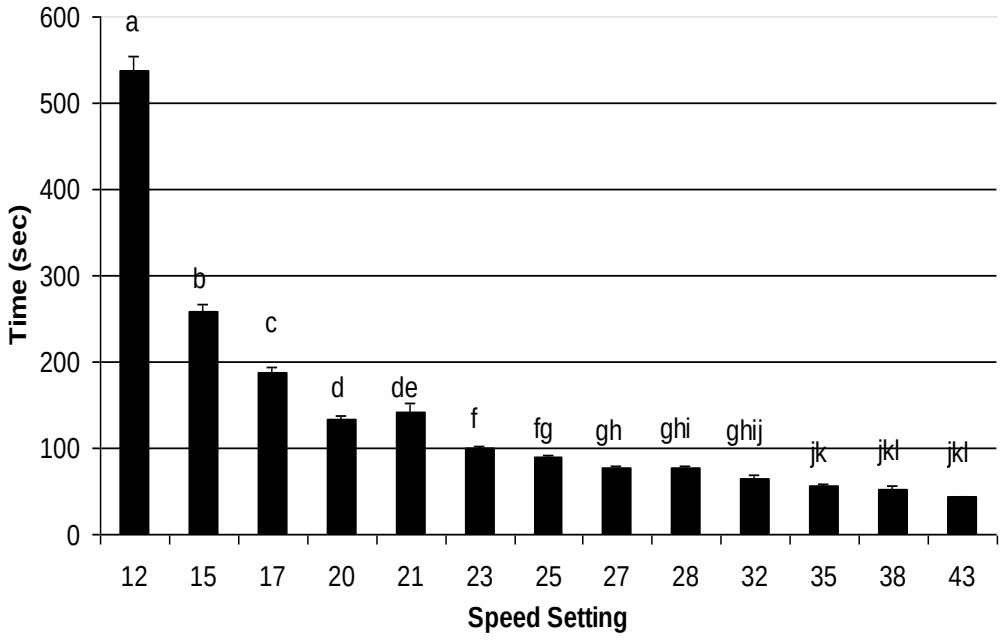

Figure 3 Average time for product to move through the spray water region to the end of the Stanfos™ Trim Pasteurizer at different settings for the speed of the conveyor belt. Different letters indicate a significant difference in time ($P<0.05$). Error bars represent the SEM.

product to move through the Stanfos™ Trim Pasteurizer at the lower settings (12-20) were significantly different from each other, while at intermediate settings (23-28), there were no significant difference in time among several settings (Figure 3). Setting 25 was not significantly different (P>0.05) from 27, 28, and 32, while setting 27 was not significantly different (P>0.05) from 28 and 32. There were no significant differences in pasteurization times at high settings (32, 35, 38, and 43). These data show that at lower speeds (below 20), small adjustments in the settings had a significant impact on pasteurization time; however, at higher speeds one setting adequately represented the group of higher settings.

The times to reach the different pasteurization temperatures for the sliced bologna, turkey breast and roast beef are shown in Table 3. The sliced roast beef reached the target temperatures faster than either the bologna or turkey breast slices.

Table 3 Times (s) for vacuum skin packaged sliced bologna, turkey breast and roast beef to reach different temperatures in the Stanfos™ Trim Pasteurizer.

Temperature (°C)	Time (sec)		
	Bologna	Turkey Breast	Roast Beef
60	78 ± 3.8[1]	82 ± 3.6	46 ± 1.5
65	103 ± 7.8	107 ± 6.8	59 ± 1.6
70	150 ± 5.3	158 ± 5.2	80 ± 1.8
75	272 ± 13.0	295 ± 4.8	149 ± 3.1
80	533 ± 15.5	720 ± 31.7	200 ± 5.8

[1]Standard error

Due to the large size (11cm length, 2.1cm width) of the sausages, initial tests were performed to determine if sufficient temperatures for bacterial inactivation could be reached using the Trim Pasteurizer. Although a final temperature of 80°C was reached in all packages, the time required differed greatly with probe location. Times of 40, 35, and 18 min were required to reach 80°C with the probe located between the two inner sausages, on the top surface, and on the bottom surface, respectively (Figure 4). These times to reach 80°C were similar to the ones found by Huang, et al. (2006) in which 2 cm wide beef frankfurters were tested. The smoked sausages took longer time to heat to 80°C compared to all other products used in this study. This experiment showed that products of larger thicker products, such as sausages, are not an appropriate product to be pasteurized with the Trim Pasteurizer as the process is too slow for efficient production as compared to thinly sliced shingled RTE meat products. The long pasteurization times would not be feasible for use in a small processing facility as production would slow significantly. As a result, sausages were omitted from subsequent microbiological challenge experiments.

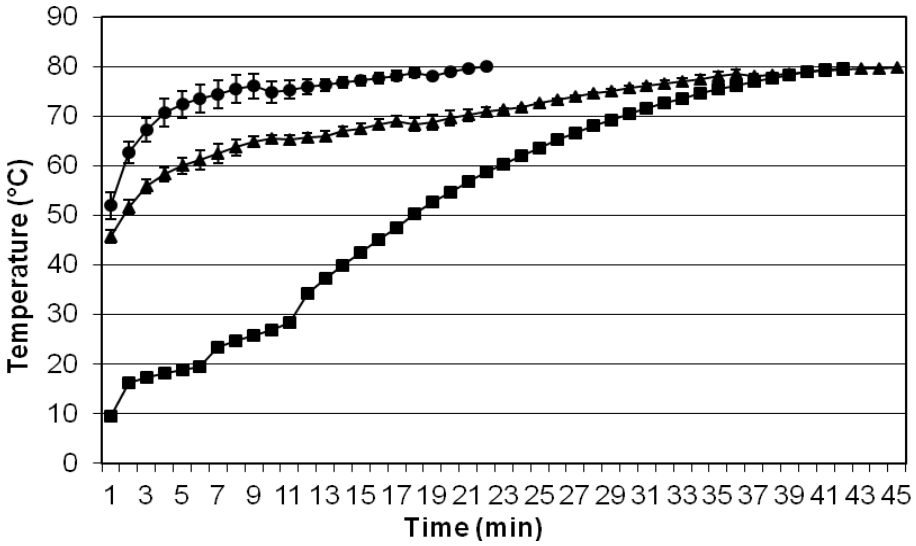

Figure 4 Heating time of smoked sausages in a Trim Pasteurizer with the thermocouple located at the top surface of sausages (▲; n=3), on the bottom of sausages (●; n=3), and between two middle sausages (■; n=2).

3.2 Reduction of bacteria on RTE meat products after pasteurization

Mesophilic aerobic bacteria and presumptive lactic bacteria were enumerated after pasteurization on all three RTE meat products that had not been inoculated with *L. monocytogenes* to determine the effect of pasteurization on the background microflora. Results for microbiological analysis of bologna are shown in Figure 5 after 48 hours. For pasteurization temperatures up to and including 70°C, the log reduction of aerobic bacteria (determined on PCA) was significantly higher ($P<0.05$) on the samples with both sides exposed to heat than on either the samples where only one side of the product was exposed to heat or the samples taken from the middle slice. The reduction in numbers of presumptive lactic acid bacteria were not different between the samples taken from slices that were exposed to heat on both or one side at 65°C and 70°C. The two highest

temperatures tested (75 and 80°C) had the greatest effect on reduction of both aerobic bacteria and presumptive lactic acid bacteria, with reductions of approx. log 4.5 for both bacteria groups at all sampling locations (Figure 5).

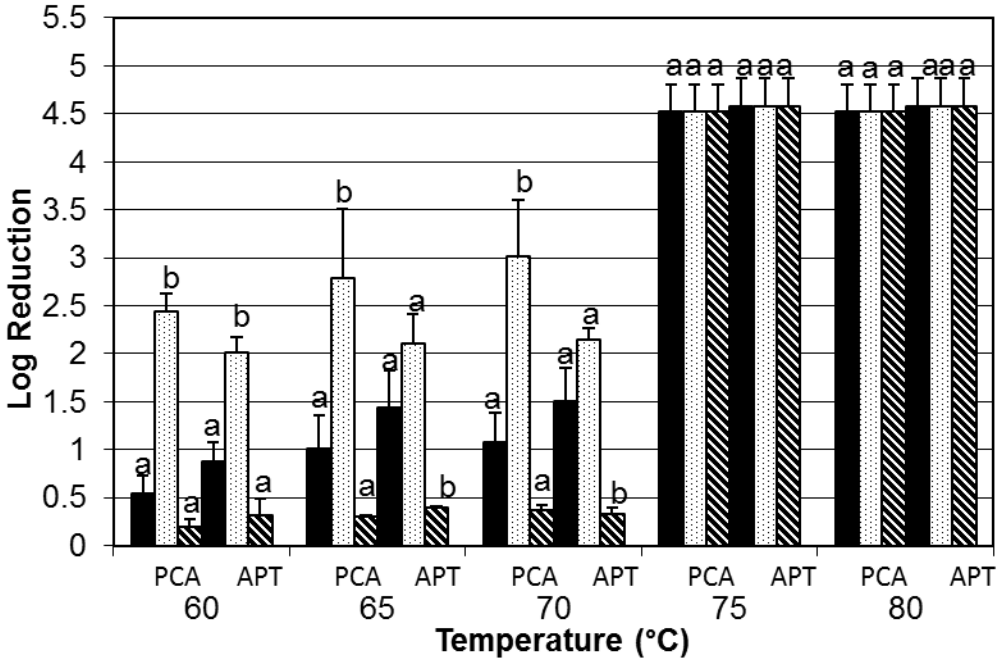

Figure 5 Reduction of aerobic mesophiles (determined on PCA) and presumptive lactic acid bacteria (determined on APT) on inoculated sliced bologna treated by post-packaging pasteurization to different internal temperatures. Black bars represent samples taken from areas exposed to heat on one side, dotted bars represent samples taken from slices exposed to heat on both sides, and hatched bars represent samples taken from middle slices. Error bars represent SEM, n=3. For each temperature and media, bars with different letters are significantly different ($P<0.05$).

Log reductions of aerobic and presumptive lactic acid bacteria on inoculated sliced turkey breast are shown in Figure 6. Counts of both aerobic and presumptive lactic acid bacteria were TNTC on one replicate; thus, results are the mean from the two remaining replicates. Reduction of both mesophilic aerobic

bacteria and presumptive lactic acid bacteria was highest on the slices exposed to heat on both sides at lower pasteurization temperatures, while the reduction on slices exposed to heat on one side and middle slices were similar (Figure 6). The two highest temperatures tested produced the greatest reduction in both types of microflora, with a 5-log reduction observed at all sample locations at 80°C. This would exceed CFIA's requirement for a minimum 3-log reduction for a post lethality treatment (CFIA, 2011).

On inoculated sliced roast beef, there were no significant differences between reductions of aerobic or presumptive lactic acid bacteria at any of the sampling locations at any temperature (Figure 7). Higher temperatures (70-80°C) produced greater reductions of both bacteria groups than at 60 or 65°C.
Sliced bologna and turkey breast had similar patterns of reduction of both aerobic and presumptive lactic acid bacteria. At temperatures from 65-70°C, samples taken from slices exposed to heat on both sides had the highest reduction, followed samples taken from slices exposed to heat on one side and samples taken from the middle slices (Figures 5 and 6). Reductions on sliced roast beef did not follow the same trends at the lower pasteurization temperature of 60°C and 65°C. For sliced roast beef, there were no significant differences among the samples taken from the different locations at any temperature (Figure 7). Higher temperatures (75 and 80°C) reduced counts by at least 4 log units on bologna and turkey breast, while on roast beef, 70°C was sufficient to reduce the counts by the same value (Figure 7). The pasteurization residence times for roast beef at all temperatures were also shorter than for bologna and turkey breast (Table 3).

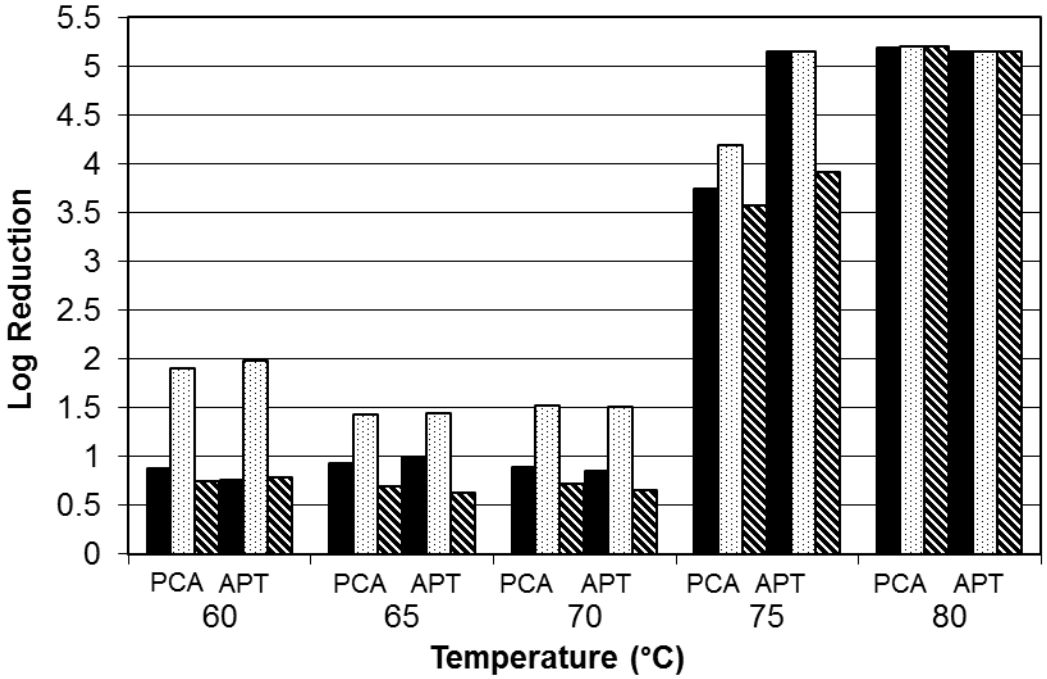

Figure 6 Reduction of aerobic mesophilic bacteria (determined on PCA) and presumptive lactic acid bacteria (determined on APT) on inoculated sliced turkey breast treated by post-packaging pasteurization at different temperatures. Black bars represent samples taken from areas exposed to heat on one side, dotted bars represent samples taken from slices exposed to heat on both sides, and hatched bars represent samples taken from middle slices. (n=2).

Other researchers found similar log reductions while using a submersed water PPP process. Selby et al. (2006) reported that samples of bologna 5 min at 65°C to achieve a 3 log reduction which is similar to the results of the current study obtained for the samples taken from slices exposed to heat on both sides.

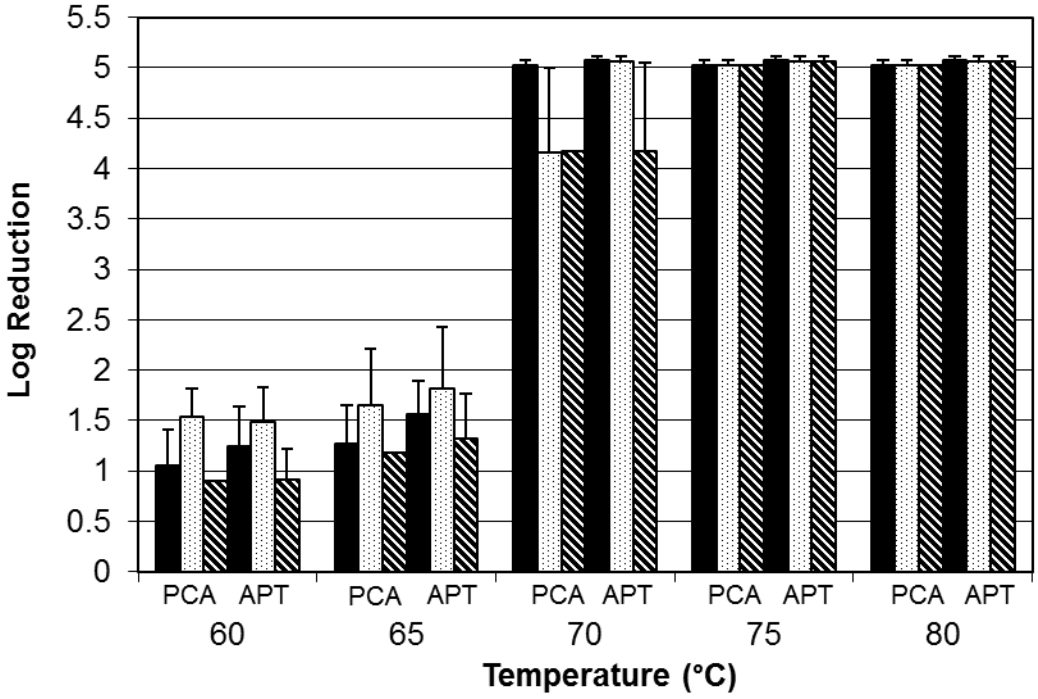

Figure 7 Reduction of aerobic mesophiles (determined on PCA) and presumptive lactic acid bacteria (determined on APT) on inoculated sliced roast beef treated by post-packaging pasteurization at different temperatures. Black bars represent samples taken from areas exposed to heat on one side, dotted bars represent samples taken from slices exposed to heat on both sides, and hatched bars represent samples taken from middle slices. Error bars represent the SEM, n=3.

Other studies on PPP of RTE meat products (Muriana et al., 2002; Murphy, & Berrang, 2002; Muriana et al., 2004) used higher pasteurization temperatures (>80°C) as the objective was to test the effectiveness of a PPP process and the related reduction of *L. monocytogenes*, but did not address the accumulation of purge or appearance of the product after pasteurization.

Reduction of *L. monocytogenes* on sliced bologna, turkey breast, and roast beef after pasteurization are shown in Figures 8, 9, and 10, respectively. In general, the trend in the reduction of *L. monocytogenes* was similar to the trend of

aerobic mesophilic bacteria for bologna and sliced turkey breast. At pasteurization temperatures of 60°C, 65°C, and 70°C, reduction of *L. monocytogenes* on samples of bologna and turkey breast from slices exposed to heat on one side and on samples taken from middle slices were not significantly different from each other ($P>0.05$), but both were significantly lower ($P<0.05$) than the reduction achieved on slices exposed to heat on both sides. On slices of bologna exposed to heat on both sides, the reductions of *L. monocytogenes* were approximately 2.5 log units

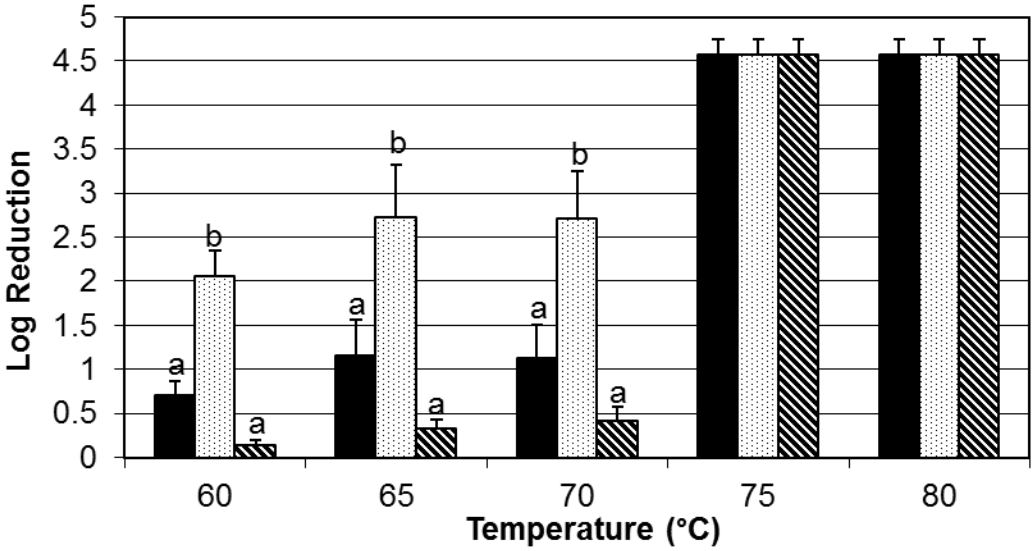

Figure 8 Reduction of counts of *L. monocytogenes* on inoculated bologna treated by post-packaging pasteurization at different temperatures. Black bars represent samples taken from areas exposed to heat on one side, dotted bars represent samples taken from slices exposed to heat on both sides, and hatched bars represent samples taken from middle slices. Error bars represent SEM of n=3. For each temperature, bars with different letters are significantly different ($P<0.05$).

for samples pasteurized to 60-70°C (Figure 8), while on sliced turkey breast, a 1.5-log reduction was observed (Figure 9). For roast beef, there were no significant differences in log reduction between sampling regions at any temperature (Figure 10). For roast beef and bologna, temperatures above 70 or

75°C were sufficient to eliminate *L. monocytogenes* on the day of pasteurization, but temperatures higher than 75°C were required for the same reduction on turkey breast slices.

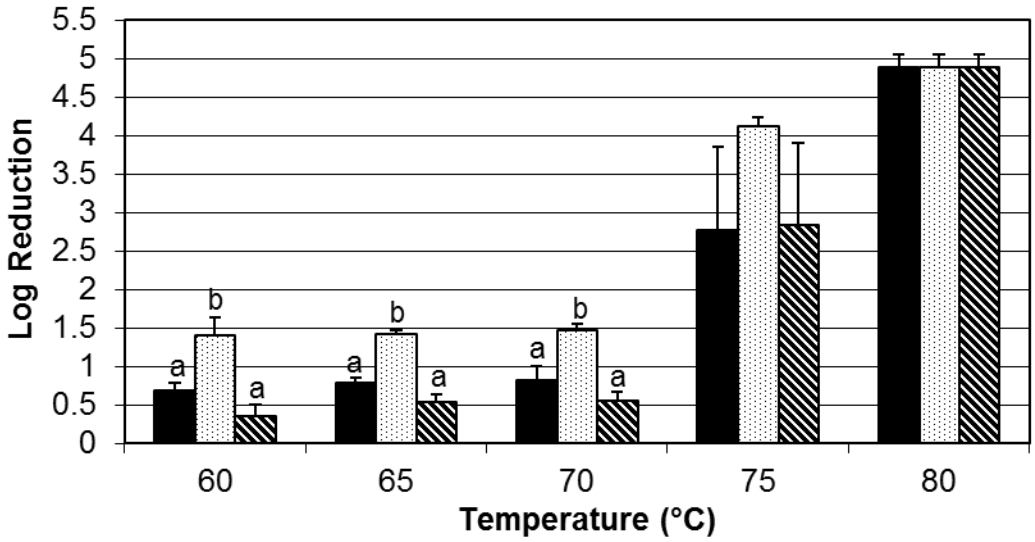

Figure 9 Reduction of numbers of *L. monocytogenes* on inoculated sliced turkey breast treated by post-packaging pasteurization at different temperatures. Black bars represent samples taken from areas exposed to heat on one side, dotted bars represent samples taken from slices exposed to heat on both sides, and hatched bars represent samples taken from middle slices. Error bars represent SEM of n=3. For each temperature, bars with different letters are significantly different ($P<0.05$).

Sampling location had an effect on survival of mesophilic aerobic bacteria, presumptive lactic acid bacteria, and *L. monocytogenes* on bologna and sliced turkey breast. Such results indicate that a heating gradient may occur within such products, as slices that were exposed to heat on both sides had a higher log reduction than slices exposed to heat on one side or slices from the middle of the package. This is likely due to the combined exposure of the slices to both the steam and the hot water increasing the product's surface temperature.

As the product moves through a pasteurizer, exposed slices are heated quickly, while heat travels slowly from the surface towards the interior (Mangalassary et al., 2004). To achieve product

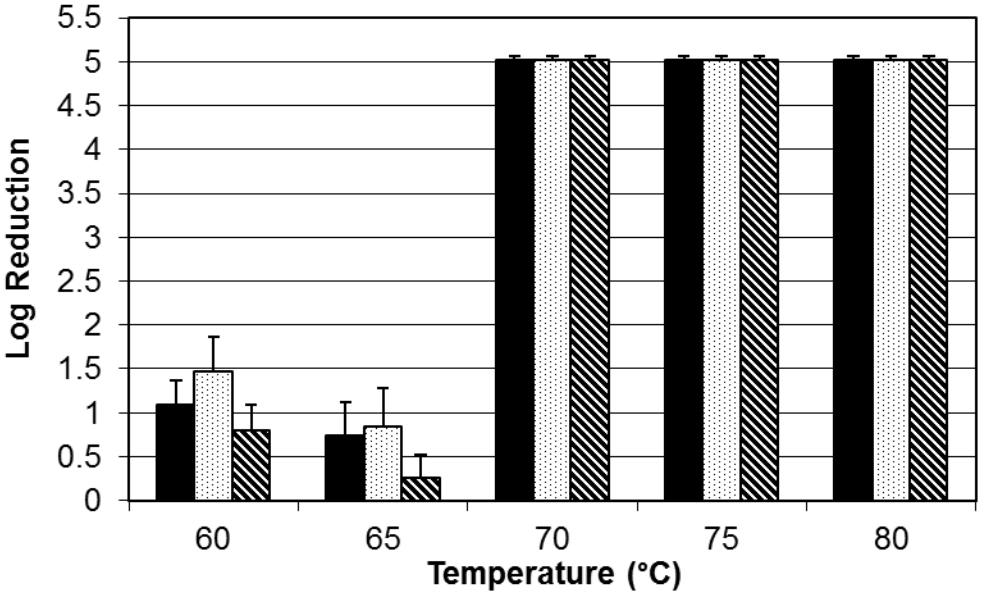

Figure 10 Reduction of heat-resistant *L. monocytogenes* on inoculated sliced roast beef treated by post-packaging pasteurization at different temperatures. Black bars represent samples taken from areas exposed to heat on one side, dotted bars represent samples taken from slices exposed to heat on both sides, and hatched bars represent samples taken from middle slices. Error bars represent SEM of n=3.

internal temperatures of 60°C, 65°C, 70°C, a shorter exposure time inside the Trim Pasteurizer was required, but this was not sufficient to affect the overall log reduction on the slices exposed to heat on one side and in the middle of the package as these pieces are shielded by the overlapping orientation of the slices.

Unlike bologna and sliced turkey breast samples, samples taken from different locations within the sliced roast beef packages treated at 60 to 70°C did not have significantly different numbers of survivors. Roast beef was not a cured

product compared to turkey breast and bologna, and based on the high protein concentration, may have contained more free water (FSIS, 2011). During pasteurization at lower temperatures, the migration of the purge could have resulted in a better heating transfer, and no significant differences ($P>0.05$) in the log reduction for the three sliced regions. Differences in thermal properties, product composition, product thickness or orientation may account for some variation but for all products, the target internal temperature was consistent.

It was entirely possible that the orientation of the slices in the package would lead to a greater log reduction for the slices exposed to heat on one side compared to the middle pieces, but this was not the case. Because the temperature throughout the Trim Pasteurizer was uniform, it was presumed that both the top and bottom of packages received the same thermal treatment and should have resulted in similar trends in reduction of bacterial counts. However, the temperature profiles for the sausages (Figure 4) demonstrated that even with consistent temperatures in the Trim Pasteurizer, the heating source (water spray valves compared to water in the pasteurization tank) may change the rate of heating. Since the bottom of the sausage heated faster than the top layer, the exposure to the hot water in the pasteurization tank had a greater conductive heating effect compared to exposure from the spray valves.

3.3 Purge formation on the day of pasteurization and after storage

Purge from packaged meat products is unappealing to consumers; thus, new processes such as PPP must be evaluated for the production of purge. To

determine the extent of product purge in pasteurized packages of RTE meats, purge was measured on day of pasteurization and after four weeks of storage. For sliced bologna (Figure 11) and turkey breast (Figure 12), there were no significant differences between amount of purge on the day of pasteurization and after storage for samples pasteurized to 60, 65 and 70°C. However, there were significantly lower amounts of purge in packages pasteurized at 75 and 80°C after storage for four weeks. It was obvious that during storage, purge receded back into the product. For roast beef, there were no significant differences between purge on the day of pasteurization and after four weeks of storage in packages treated at any pasteurization temperature (Figure 13).

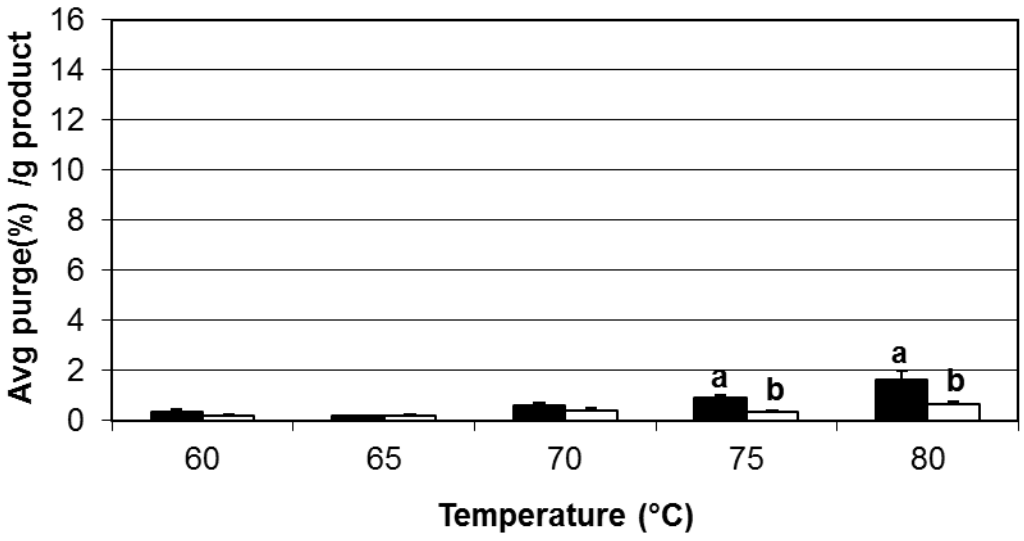

Figure 11 Average amount of purge in packaged bologna on the day of pasteurization (black bars) and after four weeks of storage at 7°C (white bars) for each of the different pasteurization temperatures. Error bars represent SEM of n=6. Bars with the different letters are significantly different ($P<0.05$).

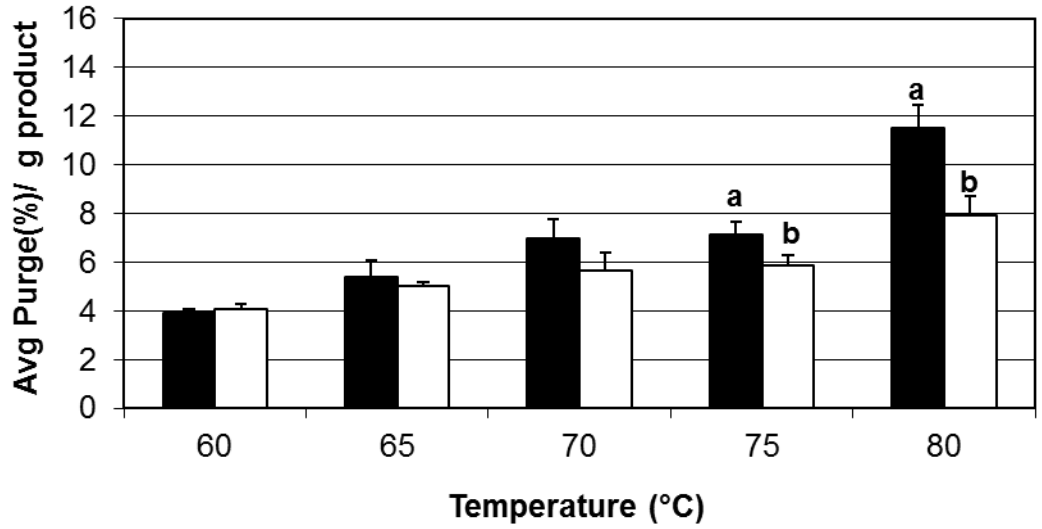

Figure 12 Average amount of purge in packaged sliced turkey breast on the day of pasteurization (black bars) and after four weeks of storage at 7°C (white bars) for each of the different pasteurization temperatures. Error bars represent SEM of n=6. Bars with different letters are significantly different ($P<0.05$).

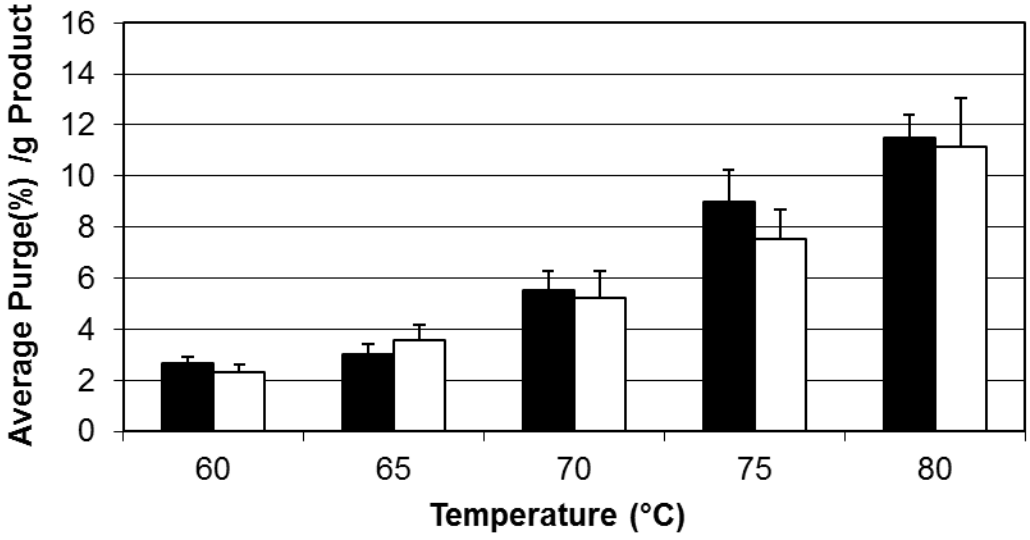

Figure 13 Average amount of purge in packaged sliced roast beef on the day of pasteurization (black bars) and after four weeks of storage at 7°C (white bars) for each of the different pasteurization temperatures. Error bars represent SEM of n=6. Bars with different letters are significantly different ($P<0.05$)

Of the three RTE meat products tested, packaged bologna produced the least amount of purge when tested directly following pasteurization regardless of pasteurization temperature (Figure 14). For pasteurization, the amount of purge in packages of turkey breast and roast beef were not significantly different (P>0.05) for any temperature. The amount of purge found in the packages in the current study is similar to that found by other researchers who applied PPP processes to different products (Ingham et al., 2005; Muriana et al., 2002).

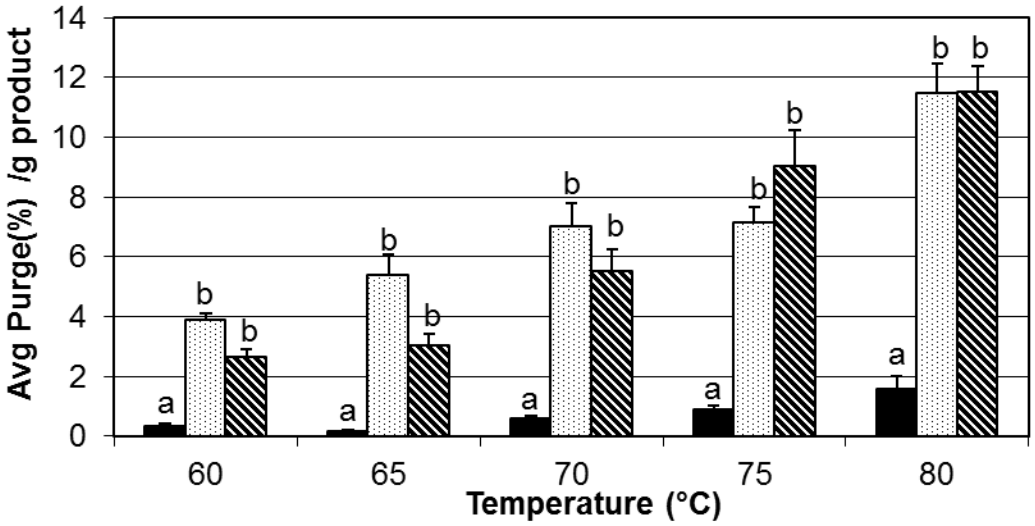

Figure 14 Average amount of purge for bologna (black bars), sliced turkey breast (dotted bars), and sliced roast beef (hatched bars) on the day of pasteurization at different temperatures. Error bars represent SEM of n=6. Bars with the same letters are not significantly different (P>0.05)

Purge in RTE meat products stored for four weeks showed the same trends (Figure 15), with bologna having the least amount of purge regardless of pasteurization temperature. Purge was not noticeable in sealed packages for roast beef and turkey breast slices after storage, but was quite evident and unappealing once the package was opened.

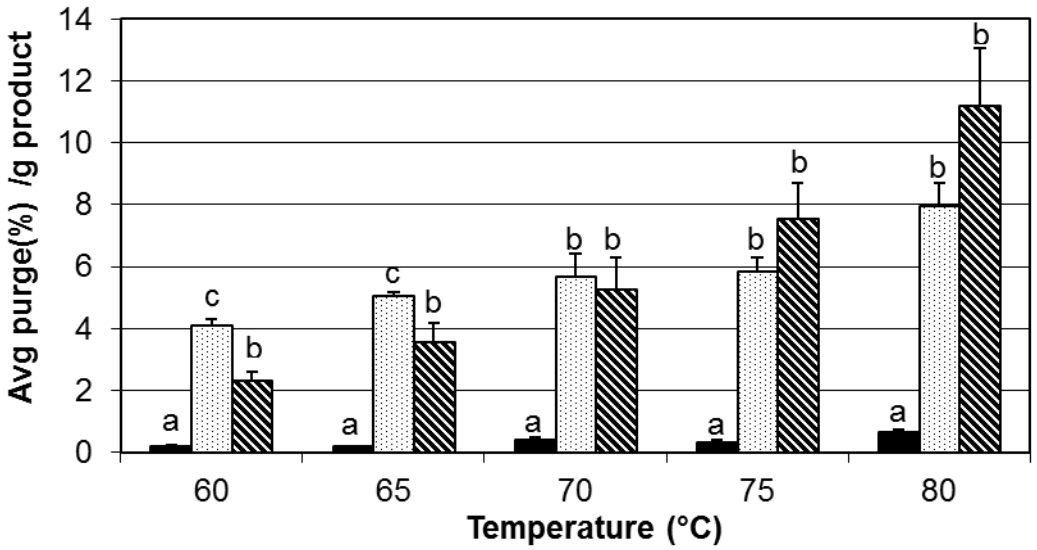

Figure 15 Average amount of purge for bologna (black bars), sliced turkey breast (dotted bars), and sliced roast beef (hatched bars) pasteurized at different temperatures and stored for four weeks at 7°C. Error bars represent SEM of n=6. Bars with the same letters are not significantly different (P>0.05)

Meat products that have a higher protein concentration compared to the amount of fat have a higher water holding capacity (FSIS, 2011), which means that less water is available for production of purge. Processed meat products tend to have a higher fat concentration relative to protein (Aberle et al., 2001), which was observed in the bologna product (Table 2). The higher fat content of bologna, combined with a high salt content, and additional binders in the ingredients likely accounts for the low amount of purge observed in this product, indicating there was less free moisture.

The turkey breast and roast beef products used in this study had a higher concentration of protein relative to fat; thus, more water is likely available to produce purge. These products had similar amounts of fat, protein, and salt,

which may be the reason that the average amount of purge was not significantly different at higher pasteurization temperatures. At the lower temperatures, significant differences between the two products could have been due shorter residence pasteurization time (Table 3). Bologna required a longer pasteurization time to achieve a final temperature of 80°C compared to roast beef, but due to the added binders in this product, less purge was produced as compared to the greater amounts of purge produced at higher pasteurization temperatures for roast beef and turkey breast. This may be due to the structural differences between whole muscle products being moisture enhanced and containing more free water being released as purge after pasteurization (Z. Pietrasik, personal communication). Roast beef was not a cured compared to turkey breast, and did not contain any sodium nitrite or sodium erythorbate, which may have resulted in greater purge formation on day of pasteurization as compared to the sliced turkey breast. During pasteurization of whole muscle products, meat proteins are denatured causing structural changes which increases the amount of purge formed. The denaturation of the meat proteins reduces the water holding capacity and may have resulted in less purge receding back into the product after storage (Z. Pietrasik, personal communication).

3.4 Recovery of *L. monocytogenes* after storage

The number of *L. monocytogenes* in inoculated bologna and roast beef pasteurized to final temperatures of 75°C and 80°C was below the detection limit (100 CFU/g) on the day of pasteurization, and was absent after 4 weeks of storage

at 7°C. In inoculated turkey breast, the concentration of *L. monocytogenes* on the day of pasteurization was below the detection limit when pasteurized at 75°C, but following storage for 4 weeks, the level had increased to >900 CFU/g for six out of the eight packages sampled after storage. This result exceeds the CFIA regulatory requirement which states that for a 125 g composite sample (5 x 25 g) after an enrichment process, *L. monocytogenes* cannot be detected (CFIA, 2011). This indicates that for sliced turkey breast the 75°C pasteurization parameters (time and temperature) was not sufficient. Pasteurization to 80°C inhibited *L. monocytogenes*, as counts were below the detection limit on the day of pasteurization and were not detected after 4 weeks of storage at 7°C.

If *Listeria* is exposed to sublethal temperatures between 44- 48°C (Farber and Peterkin, 1991) before a higher final temperature, the organism has the potential to have a higher heat resistance. Farber and Brown (1990) found that an increase in holding time at high temperatures also results in greater heat resistance in *Listeria* and a fast rate of heating (heat to 60°C at > 5°C/min) does not induce thermotolerance, but at slow rates (0.7°C/min), thermotolerance increases. The slower conveyor belt speed (Table 3) used for the sliced turkey breast could have resulted in greater thermotolerance leading to *Listeria* growth and survival after 4 weeks of storage. If the Trim Pasteurizer is to be used by processors to reduce *L. monocytogenes* to below detectable limits, more research must be done to determine the heating rate to reach target temperatures for each product. The rate at which a product reaches a final temperature when exposed to the hot water in the tank compared to the spray values is important. Because the *Listeria* strains

used for this study were relatively heat resistant, exposing the organism to sublethal temperatures with slow rates of heating may have increased the overall thermotolerance of the *L. monocytogenes*.

One possibility for the difference among the survival of *L. monocytogenes* on the products used in the current study, is that purge formed after pasteurization could have migrated from the product interior to the product surface during storage (Muriana et al., 2002). If the interior was not heated to a sufficient temperature for bacterial inactivation, the chilled purge could serve as a growth media for any remaining *L. monocytogenes*, resulting in detection of viable cells after storage. However, the amount of purge present after storage in packages of sliced roast beef and turkey breast was not significantly different ($P>0.05$), and the amount of purge in the packages of turkey breast was significantly higher ($P>0.05$) on day of pasteurization than after storage. Since *L. monocytogenes* was absent at the same pasteurization temperature in roast beef, it is likely that slice size and thickness played a greater role than the amount of purge in the packages. Another possibility is the compositional difference between roast beef and turkey breast slice.

Chapter 4: General Discussion and Conclusions

In this study, a Trim Pasteurizer was evaluated for application as PPP process to provide small to medium sized processors with a potential intervention to reduce the numbers of *L. monocytogenes* on RTE meats. The main advantage of PPP is the potential for elimination of *L. monocytogenes* in situations where contamination of cooked RTE products from equipment, workers, personal protective equipment and the processing environment can occur. The stringent sampling and testing required by CFIA for both food contact surfaces and finished product testing (CFIA, 2011) motivates the need for an inexpensive alternatives to high pressure processing for PPP of RTE meats. The addition of a post-lethality process like PPP will reduce the amount of testing required to meet the new *Listeria* guidelines, which will reduce the costs of sampling and sample analysis. Options such as high hydrostatic pressure processing may be too expensive for small processors as the capital and operating costs are high. Consumers are more health conscious now and are generally opposed to the addition of preservatives to products (Agriculture and Agri-Food Canada, 2005). Antimicrobials, such as sodium lactate, sodium diacetate, bacteriocins or bacteriophage, could be added to RTE products to inhibit the growth of or kill cells of *L. monocytogenes*.

The first objective of this study was to determine if PPP at temperatures from 60-80°C eliminated *L. monocytogenes* on sliced bologna, turkey breast, and roast beef, and on smoked sausages. Preliminary experiments determined that the

temperature increase produced by PPP was not rapid enough for practical use of this process for PPP of smoked sausages.

In the three remaining products, higher pasteurization temperatures achieved log reductions of 4.6 to 5.1 of *L. monocytogenes*. Thus, at higher temperatures the small pasteurization unit provided an effective process for inactivating *L. monocytogenes*. These reductions are greater than those reported by Muriana et al. (2002) who achieved reductions of 2 to 4 logs in numbers of *L. monocytogenes* on RTE turkey, beef, and ham treated at 90 to 96°C water bath for 2–10 min. In the current study, PPP at lower temperatures did not achieve the same reduction of *L. monocytogenes*; at temperatures of 60 to 70°C, reduction of *L. monocytogenes* varied by location of sample within the package in some products. Samples taken from slices exposed to heat on both sides of the packages had greater reduction of *L. monocytogenes* than either slices exposed to heat on one side or samples taken from slices in the middle of the package. It appears that the shielding effect of overlapping pieces may play a role at lower temperatures, but not at higher pasteurization temperatures. Also, residence pasteurization times were shorter in roast beef compared to bologna and turkey breast, which may play a large factor for a processor in deciding the most viable RTE meat product for the pasteurizer. Mangalassary et al., (2004) reported that given enough time, the entire product accumulates enough heat for the surface and interior parts to reach the same final temperature, which explains why at higher pasteurization temperatures, the log reduction was similar regardless of the sampling location. A

better thermal profiling method is needed to determine the heating exposure in each region in the package so that a more efficient PPP process can be designed.

The second objective of the study was to determine the purge formed in RTE products following PPP. Purge formation was greatest for roast beef and turkey breast slices on the day of pasteurization and after storage, while sliced bologna had the lowest amount of purge formed at the time of pasteurization and after storage at all sampling times. Purge formation increased with pasteurization temperature, with the most purge formed at 80°C.

Overall, PPP with the Trim Pasteurizer from Stanfos Inc. is a suitable process for small RTE meat processing after taking into consideration the thickness and uniformity of the product. A stacked bologna product due to the product thickness may be as impractical as the sausage product. Sliced shingled product is the most useful application for the equipment; however, size and thickness of packages are important variables that must be considered. At higher processing temperatures, formation of large amounts of purge was a problem. The purge in a package can act as a growth medium for *L. monocytogenes*. However, at lower pasteurization temperatures, there was no significant increase in amount of purge after four weeks of storage. The amount of purge produced after pasteurization for bologna was significantly less for all temperatures compared to turkey breast and roast beef product. It was difficult to notice the purge in packages of bologna after pasteurization to 75 and 80°C until after the package had been opened, but since a quarter of consumers will judge a product by the appearance (Kosa et al., 2007a), consumers may be reluctant to purchase this

product again. Optimally all the products should be pasteurized to 75°C to meet regulatory requirements for all sections of the product regardless of the orientation of the slices in the package. However, there were noticeable amounts of purge in the unopened packages of roast beef and turkey breast slices, which would not be appealing for consumers. Thus, the Trim Pasteurizer is a suitable PPP process for eliminating *L. monocytogenes* in shingled uniform thick sliced RTE meats, but due to the amounts of purge produced at suitable temperatures (75°C), it would not be a feasible option for a small meat processing company unless products are formulated to ensure that the water binding of the product is sufficient to prevent accumulation of purge in the packages.

4.1 Future work

Although PPP is a promising technology for reducing *L. monocytogenes* in RTE meats, further work must be done to optimize the process. It is important to determine the time-to-cost ratio for the processors. Another consideration is the thickness and orientation of the slices in the package. The Trim Pasteurizer may not be a feasible option for pasteurization of thick RTE products (i.e. sausages) or products packaged in stacks of slices, as the processing time required may be too long for production efficiency. This research found that sliced bologna, turkey breast, and roast beef were suitable for treatment as long as the product is packaged in a shingled orientation, while smoked sausages were not a feasible option. Thus, future work needs to be done to determine the maximum thickness of a RTE product for efficacy.

Another factor that must be investigated is development of a more accurate method to measure the final temperature of the product exiting the Trim Pasteurizer. When measuring the final temperature as the package exits the Trim Pasteurizer, only a small section of the product is measured with a thermocouple probe head before the product begins to cool. Shifting the thermocouple within the package produced different temperature readings; thus, undetected cold spots may also exist within the product. An optimal method would be to have an infrared thermal sensor that displays the heating gradient across the entire product, which would give a better understanding of differences in heating by spray values, hot water in the Trim Pasteurizer tank, and steam. Challenges to this technology include steam and water on the package preventing accurate infrared thermal measurement (Glockmann, 2012). The use of thermal topography would give more realistic information about the entire pasteurization process and determine if the product is being uniformly heated.

Appearance plays an important factor in purchase of any deli meat product and reduction of purge formation is crucial for consumer acceptance. If the temperature of pasteurization is sacrificed for reduction of purge in the package, then inclusion of other alternatives methods to reduce growth of *L. monocytogenes* is required. Alternatives could include a prepackage and postpackage method (Muriana et al., 2004) that results in no purge accumulation in the package. The addition of a surface antimicrobial such as nisin in combination with lysozyme and PPP can result in a 4-log reduction in numbers of *L. monocytogenes* using pasteurization temperatures of 62.5 and 65°C

(Mangalassary et al., 2007) could be considered to inhibit growth or inactivate *L. monocytogenes* while minimizing the amount of purge that accumulates in the package.

A combination of methods that could reduce the temperature needed for pasteurization while still providing a sufficient level of kill or inhibition of *L. monocytogenes* during storage would allow the use of the Trim Pasteurizer by smaller processors.

Chapter 5: References

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